ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

FISCAL YEAR 1971

PART I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

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NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

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NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

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ANNUAL REPORT

July 1, 1970 through June 30, 1971

National Institute of Neurological Diseases and Stroke
National Institutes of Health

The Director's Report

In 1971, the Institute continued to support a balanced research program in which clinical and non-clinical studies were about equally represented. A small increase in funding of research grants gave some relief to grantees whose budgets have been subjected to arbitrary renegotiation and provided for feasibility and pilot studies to initiate the development of acute Spinal Cord Injury Research Centers. This major new program will begin in 1972. On the other hand, inflation, mandatory salary increases, and the continuing decrease in allowed budget and positions caused a decrease in the total program of the Institute.

Particularly serious is the steady decline in training activities supported by the Institute. Twenty-six training programs were discontinued in 1970, ten more in 1971, and it is estimated that by the end of 1972 a total of 56 programs will have been dropped for lack of funds, and the remainder held to an 85 percent operating level.

In the early 1950's, when NINDS started its training programs, neurology, otolaryngology, and ophthalmology were essentially static disciplines. Teaching was mainly at the bedside and little effort was being made to find better ways to treat and prevent neurological and sensory disease. In medical neurology it was necessary for the physician to go to Canada or Europe for adequate postdoctoral training in these areas.

The impact of our training programs has been to introduce a science state into the medical training in our fields of responsibility. This has brought physiologists, biochemists, and anatomists into clinical teaching departments and has provided for both the clinical faculties and the trainees during the time they engage in research, training and other activities not chargeable to patient care. As a result the Nation now has a core group of superior teachers and clinical investigators who are both capable of initiating the development of new methods for prevention and cure, and who are quick to understand and apply methods developed in other specialties to the special problems of neurological and communicative disorders.

In addition, badly needed sub-specialties such as pediatric neurology and neuroradiology are being developed by our training programs, traineeships and special fellowships. Fellowships are also being used to permit clinical investigators to spend full time in learning new disciplines and techniques which they can apply in their research, and in interesting physiologists, pharmacologists, anatomists, and other bioscientists in neurological and communicative disorders.

The discontinuation of clinical training programs and the reluctance of Medical School Deans to provide funds to support training in what are considered minor specialties are forcing many faculty members to abandon research. In order to support themselves they are being forced to turn to full time practice. If this trend is not reversed, the quality of training can be expected to return to the pre-1950 level. Furthermore, if the 1968 level of training were maintained, it is estimated that even this would produce only one-half of the teachers required to train the number of neurologists and otolaryngologists needed by 1985.

In 1971, Dr. Warren Huber, Chief Neurologist, Veterans Administration, and member of the NANDS Council, joined the Institute as Associate Director for Collaborative and Field Research. Under his leadership a collaborative study of the proposed criteria for cerebral death was planned. This study will start during FY '72.

Since the data collection phase of the Collaborative Perinatal Study is now largely completed, the emphasis in this project is now on data analysis. To meet these changing requirements, it is now planned to reorganize the Perinatal Branch and to transfer the Office of Biometry from OD to C&FR.

In addition, noteworthy progress has been made in virus studies, anticonvulsant drug testing, and the sensory prosthesis program. In intramural research tremendous progress has been made in the diagnosis, even before birth, of a group of hereditary lipid-storage disorders, most of which cause severe mental retardation. Tests for unaffected carriers of the defective gene and of the unborn fetus have been developed for nine of these diseases so that there is now an excellent prospect that these disorders can be eliminated from the population within the foreseeable future.

Two important clinical developments have come to fruition in 1971. Alternate day steriod treatment developed in the Medical Neurology Branch has been found effective in a considerable number of patients suffering from myasthenia gravis, and acetazolamide has been shown to be effective for periodic hypokalemic paralysis.

Transfer of administrative responsibility for the primate colonies to the University of Puerto Rico has been completed. Under the able administration of Dr. Clinton Conoway, productive research is being carried out at an accelerated pace.

During FY '71, two resident conferences were held to acquaint supervisory personnel with EEO problems and to develop techniques for their solution. To further the aims of the EEO programs, the Institute EEO Advisory Committee has met regularly with the Director and his staff, several meetings of women and minority groups have been held, and a task force under the Deputy Director has formulated an affirmative action plan. The Executive Officer, Personnel Officer and their staffs have initiated a major and continuing effort to implement the affirmative action plan. In addition, the Director has established an Open Door Policy, making himself available whenever possible between 8:45 and 9:15 a.m. to discuss EEO problems confidentially with any employee.

As a means to recruit and train more qualified black people in the neurosciences, a program entitled Frontier in Research and Teaching in Neurosciences was established under a grant to the Marine Biological Laboratory of Woods Hole, Massachusetts. Several trainees from predominantly black colleges are currently in the program. It is hoped that this project will serve as a model for many more similar summer projects.

A device to calibrate and test automatically all channels of EEG apparatus used in the evaluation of cerebral death has been developed by the Director in collaboration with the Technical Development Section, NIMH-NINDS.

Edward F. MacNichol, Jr., Ph.D.

The Office of Public Information

The Office of Public Information of the NINDS organizes and carries out a comprehensive public information program to inform the general public and specific individuals and groups about the programs and operations of the Institute. Information activities are designed to achieve greater understanding and appreciation of NINDS programs, activities and research accomplishments on the part of Congress, biomedical institutions, and the medical professions.

The NINDS Information Office activities can be grouped conveniently under the following headings: publications; press and periodicals; films, TV, and radio; internal reporting, exhibits, and public inquiries.

Public Inquiries

During the past year many more people wrote the White House and Congress. Every medical research cutback, budget change, or news story concerning cancer or other major disease brought forth a new freshet of letters. As a result, at times the Information Office staff has had to delay many of its regular activities to draft replies to the many letters from individuals and Congressional offices.

More time was spent by the Office staff responding to letters than on any other activity. The complexity and variety of subject matter raised in these letters require considerable research as well as tact in preparing replies. In replying to Congressional calls, staff members often draw on a number of sources in compiling statistics or other material. Members of the press require information which must be obtained both from the files and through calls to scientists. Patients often write to request admission to the Clinical Center or to consult with NIH research physicians. In such cases, patients are advised of the procedures to be followed.

At least 3,000 inquiries required individually prepared letters in answer to specific questions. Congressional mail was extremely heavy, and several hundred letters were in response to these inquiries. In addition, many letters were answered by Congressional staffs after information was supplied over the telephone from the Information Office. Thousands of inquiries were answered through the use of printed materials. In addition, many patients or family members phoned for information. Altogether, these requests total more than 100,000 each year.

Publications

The Information Office is divided into two sections: Public Information and Scientific Publications. The Scientific Publications section processes and publishes through the Government Printing Office many publications both for the Information Office and also for other areas of the Institute.

In the past two years, the Information Office has more than doubled services relating to production of scientific and professional publications without any increase in staff devoted to this work. The services are usually requested by various administrative units of the Institute, or by either NANDS council subcommittees or Institute committees, and usually involve responsibility for all stages of production from typewritten manuscript to editing, design, proofing of galleys and pages, checking authors, printing, and distribution. Services which have to be obtained from extra-institute sources, such as Medical Arts and Government Printing Office, are coordinated so that all published projects move forward as fast as possible.

The Monograph Series—This is a series containing neurological science contributions of Institute staff and consultants. Included are reviews, reports, nomenclature, classification and methodology aids, and proceedings of meetings sponsored by the Institute. Twelve of these monographs have been published, and another one is in the process of production. Three monographs produced this year are: Human Communication and Its Disorders (NINDS Monograph No. 10), Reading Forum (NINDS Monograph No. 11), and Studies on the Clinical Efficacy of Marketed Antiepileptic Drugs (NINDS) Monograph No. 12).

The Monograph Human Communication and Its Disorders is the product of the Council Subcommittee on Human Communication and its Disorders. Five years in preparation, this manuscript ran to 616 pages, with 66 tables and illustrations. In print, it contains more than 200 pages. The present heavy demand for this publication is expected to continue since there is now no other review volume covering this entire field.

Reading Forum consists of more than a score of background papers which were contributed by leading figures in the field to the Secretary's (HEW) National Advisory Committee on Dyslexia and Related Reading Disorders. This collection of papers offers analyses, approaches, and discussions useful to those concerned with children who do not learn to read effectively.

Monograph 12, Studies on the Clinical Efficacy of Marketed Antiepileptic Drugs, reviews some 250 studies of the thirteen presently marketed primary antiepileptic drugs, plus two compound drugs, and several combined regimens of the primary drugs. It is expected to be extremely useful to physicians treating epilepsy patients.

In the Hope through Research series, Huntington's Chorea (the 17th pamphlet in this series), was published, while several were revised. Three of these pamphlets are available in Spanish.

The latest of the Profile series, Research Profiles 1970, was published on research advances in eight major disease areas. This is a 57-page illustrated booklet of the special Congressional reports and an opening statement required by the Director during the appropriations hearings.

The Information Office has continued to assist with the publication and distribution of Epilepsy Abstracts. Beginning with Volume 4 in January 1971 there was a subscription price of $15 for Epilepsy Abstracts. The supscription receipt pays for the cost of photocomposition, printing, and mailing by the Excerpta Medica Foundation. Through a contract, the NINDS will continue to pay the cost of selection and preparation of the abstracts. The Information Office continues to handle requests for back issues of these publications.

Among other new publications during the year were: Blood Level Determinations of Antiepileptic Drugs—Clinical Value and Methods; Physical Trauma as an Etiological Agent in Mental Retardation; and A Study of New Methods of Measuring Cerebral Circulation. Several fact sheets were revised and updated. About 20 publications were reprinted in various quantities.

The Information Office is now distributing 50 publications on neurological and sensory disease problems. Of these, 32 were written for the general public on specific disorders; 18 others covering broad disease areas were prepared for distribution to the scientific community. In addition, several publications from the now defunct Neurological and Sensory Disease Control Program are being handled by the Information Office. Altogether, more than 300,000 of these publications were distributed during the past year.

Press and Periodicals

The Information Office more than doubled in the past 12 months the number of stories contributed to the agency house organ, the NIH Record. During the year, Information Office staff contributed some 70 stories to the Record, including a dozen stories for a special 20-year anniversary issue. The Office prepared 12 press releases and announcements, and assisted with two major conferences. Twenty-four articles—including both new and revised— were submitted for the NIH Research for Health series and sent to 60 small daily and weekly newspapers with a readership of 11 million.

Many of these stories received extra mileage by being run in the NIH Feature Service and the NIH News Service. The NIH Feature Service uses illustrated feature articles concerning work conducted or supported by NIH. It is distributed once a month to 100 professional journals and to newspapers through syndicates, nationally and with international potential readership of seven million.

The NIH News Service uses original news stories, specially tailored versions of press releases and other published material. It is circulated twice monthly to science and medical writers, daily newspapers, general circulation magazines, news syndicates and services, and educational and medical journals.

A special effort was made to inform the public concerning two areas of Institute research: research on epilepsy, and research and research needs in the communication disorders. Articles on these areas of research were written and accepted for publication by Parents magazine and Today's Health. With the help of the Information Office staff, a writer has contracted to publish in Good Housekeeping two stories on outstanding personalities related to neurological medicine.

A large number of reporters, free-lance and feature writers, editors and broadcasters requested information about Institute programs. The Information Office arranged interviews for representatives of the various media with Institute scientists which resulted in stories in many magazines, medical journals, and newspapers. These included: Time, Newsweek, Kiplinger's Changing Times Magazine, New York Times, Evening Star, Washington Post, Medical World News, Chemical Engineering News, Medical Tribune, U.S. Medicine, Science News, Family Health, and many others. Pictures were supplied on request to a number of magazines.

Exhibits

In response to a Department request, the Information Office has sharply reduced its exhibit activity. Exhibits were shown at only two major meetings during the year: at the AAOO in Las Vegas, and at the ASHA meeting in New York. A special publications exhibit was refurbished and displayed at a number of local meetings. Recently, the Office assisted in the desgin and construction of one small scientific exhibit.

Television, Radio, Films

An Information Office staff member arranged a number of interviews with NINDS scientists on TV and radio. Appearances were made on such shows as the WRC-TV program, "HEW and You," and on the WTTG Panorama Program. One scientist's work was discussed during a 15-minute segment of the Today Show. As a result of his appearance, the Information Office received several hundred letters and drafted replies on a number of genetic defects.

An NINDS-sponsored film produced by Churchill Films, "Early Recognition of Learning Disabilities," was selected by the Council on International Non-theatrical Events for suitability for international festival use and received a CINE Golden Eagle Certificate in the American Film Festival. In the film, daily activities of kindergarten and the first two grades are shown, and the children with learning disabilities and their problems clearly stand out in the classroom. It points out the necessity to get extra help for children who can't "keep up" and emphasizes solutions to these problems through interviews with parents.

Reports and Special Documents, Speeches and Statements

The Information Office prepared nine special Congressional reports and an opening statement required by the Director during the appropriations hearings. The Office also prepared a document, "Highlights of Research," which summarized 10 special research areas for NINDS testimony.

During the year, 56 journals were scanned on a weekly or monthly basis, and 29 research weeklies and program developments items were prepared for the Director, NIH and the Assistant Secretary for Health and Scientific Affairs using grantee or Institute scientist's findings. Arrangements were made for many Institute lectures, including the preparation and distribution of flyers.

Four speeches were written for the Director, NINDS, and the Director, NIH. Also on several occasions suggested Presidential messages and proclamations requested by leading voluntary organizations marking special events were drafted.

Special Projects

The Information Office prepared a special edition of the NIH Record celebrating the Institute's 20th Anniversary. Several thousand copies of this special edition were printed for use in answering inquiries about the Institute.

The Office prepared and issued the pilot issue of an Institute newsletter, the Instiscope. One staff member also spent considerable time writing newsletters and other material for the Equal Employment Opportunity program.

Cooperation with Voluntary Agencies, Foundations, and Professional Societies

The Information Office performs many services for the 20 national voluntary agencies and 9 professional societies in the neurological and sensory area. Staff members meet with officials of nearly all of these agencies every year. Educational and publicity campaigns are coordinated between the Institute and these agencies. Much of the literature published for the general public is distributed by them. In turn, the Information Office provides facts on budget and research, arranges for speakers at meetings, and helps with the development of exhibit programs, movies or other educational materials. Especially close cooperation was maintained with the National Committee in Neurological Disorders and its many members. A newsletter, the "NINDS Review," is published five or six times a year and distributed to officials of these organizations.

The Office of Program Analysis

The Office of Program Analysis is the focal point for in-depth information on all scientific activities of NINDS and for information about scientific activities of government and non-government agencies relating to the mission of NINDS. This information is used for the review and evaluation of the NINDS programs and for developing and assisting in the development of plans for new programs to achieve the major goals and objectives of NINDS. It is also used to answer questions concerning research on neurology, neurosurgery, sensory disorders and neurosciences quickly and accurately.

The Office of Program Analysis has many continuing activities. A cross-indexed file of all research related to the mission and goals of NINDS, whether supported by NINDS or not is maintained. This file is searchable by computer and manual means. Summaries of the total Institute effort in major program areas, combining the work carried on and supported by the various administrative parts of the Institute are annually updated. Material and reports relevant to NINDS program area are continually added to categorical program files. Special reports relevant to NINDS problems and programs are produced. Workshops on Institute problems are generated and conducted; and the Neurological Information Network and other scientific information activities of the Institute are centrally directed by the Office of Program Analysis. A roster of scientific personnel in the neurological specialties and neurosciences is maintained. The Office serves as the focal point for the international research supported by the foreign currency credit award activities (PL-480) of the NINDS and monitors this program. Each of these activities will be described briefly, giving major higlights of the past year.

File of research related to the NINDS mission.

Some 1500 odd index terms which discribe the research projects supported by NINDS (Intramural, Collaborative and Field Research and Research Grants) have been entered on Keydex cards. The research project grant number and Intramural and Collaborative and Field Research project numbers are punched on these cards which makes possible very rapid coordinated searches in considerable depth to identify current research on almost any relevant subject. The files are kept current by constant updating. The output from this manual search system is the grant or project number; for details one must consult data books or grant files.

Over the past two years a computerized base of information on ongoing research in the areas of interest to NINDS has been established and kept updated. This data base is stored for retrieval in the Division of Computer Research and Technology (DCRT) Publication Retrieval System. This data base is composed of two sets of information: 1. NINDS supported research, i.e. extramural grants, intramural, collaborative and field research projects, contracts, and 2. non-NINDS supported research in neurological and sensory disorders, both federally and privately funded. Both files contain esentially the same data items: number, disorder classification, institution, location, title, duration, investigators, summary and descriptors. The descriptors for the Institute-supported research were assigned by DRG, usually 10 to 15 per project, while the descriptors for the non-NINDS research are from the SIE (Science Information Exchange) System. All of the data items mentioned can be retrieved. This computer-based information system allows for free text search and retrieval, increasing specificity and the efficiency of retrieval. Retrieval options include a complete or partial listing of all information in the file for any particular term or terms -- author, title, journal information, abstract, indexing terms, etc.

The DCRT Publication Retrieval System which can be entered from P.A. office in Building 31 also contains the citations collected by the Parkinson Information Center and Volume I (1968) and the Retrospective Bibliography (1947-1957) of Epilepsy Abstracts.

Interaction between the Office of Program Analysis and the Extramural Programs has been developed to facilitate the exchange of information and to efficiently use existing resources of both groups. This has been especially helpful in keeping the files current, deleting terminated projects and adding new ones as they are awarded. This interrelationship with the data analysis section of Extramural Programs has allowed the storage of records in the program analysis files to be substantially reduced. Microfilming of the active NINDS research grants and projects has been discontinued with considerable saving in cost and personnel.

In addition to the file of information on ongoing research, a "categorical disease file" of published and other material relevant to the programs of NINDS continues to be maintained. This archival activity does not try to capture all relevant material, but only that which is acquired in the course of everyday work and as a result of special searches, reports, and by anticipatory action or reaction.

Relevant material in this system includes journal articles, selected because they are historical, classical, review, state-of-the-art or survey type or because current trends in basic and clinical research and treatment of any given neurological disorder can be discerned. In addition there are monographs, books, data on training programs, reports of special meetings, symposia, ad hoc committee reports and products of the Cerebrovascular Disease Alerting Service. Reprints and abstracts are perused and pertinent data selected for inclusion in the categorical file.

A meeting file continues to be maintained with a chronological list prepared twice a year for ready analysis. This list is prepared by systematically scanning the following sources:

Department of Health, Education and Welfare 18-Month
  Calendar of National Meetings
Crowell Collier and MacMillan Information Center
  World Meetings United States and Canada
Crowell Collier and MacMillan Information Center Current
  Index to Conference Papers in Life Sciences

Journal of the American Medical Association List of
  Domestic and Foreign Meetings
Office of the Director's List of Meetings
General Meetings Notices received in the mail

A bibliography of case reports of neurological diseases and reviews of neurological and sensory diseases is maintained with monthly updating from MEDLARS. This file is proving useful not only to those in the Office of Program Analysis, but also to others who are involved in research or writing about NINDS programs.

Purging of no-longer useful material from these files is a continual problem which requires considerable time.

Major user requests in the past year have been for data on:

- Accident and Injury (Spinal Cord and Head)
- Aging
- Angiography
- Bell's Palsy
- Brain Tumor Chemotherapy
- Cerebral Blood Flow Measurements
- Cerebral Palsy
- Childhood Deafness
- Congenital Malformations
- Crippled Children Data
- Criteria of Death
- Cerebrospinal Fluid Flow Research
- Economic Cost--Incidence-Prevalence Data
- Epilepsy
- Friedreich's Ataxia
- Gallium
- Hydrocephalus
- Inborn Errors
- Instrumentation--Holography
- Intelligence
- International Research
- Mental Retardation
- Multiple Sclerosis
- Muscle Research
- Parkinson's--L Dopa
- Perinatal Data
- Population Studies
- Prosthesis
  - Visual
    - subcategories
      - electrical stimulation of the visual system
      - visual physiology
      - anatomy
      - pathologic effects
      - psychology
      - biological response to materials

Prosthesis (continued)
  Visual
    subcategories
      stimulation of CNS other than visual
      effects of continuous electrical stimulation
      miscellaneous
  Auditory
    subcategories:
      same as for visual system--
      miscellaneous category has data on Noise and
      Testing of Hearing
Rheoencephalography
Short-Long-Term Memory
Stroke
Supranuclear Palsy
Ultrasonic Effects
Writer's Cramp

Library searches for published research of a given investigator, grantee, or in a given subject or program were done and bibliographies including selected pertinent articles were prepared for special reports, requests or by anticipatory action or reaction. These searches usually are of such a special nature that they include the output of several archival sources.

Medical School Faculty file.

This is a new Directory-File of medical school faculty members who are teaching in areas of interest to the NINDS. The almost completed Directory consists of a card file which includes the name, rank, discipline, location of each individual, and also a brief curriculum vitae. The system can be entered by Institution or by individual name. Information concerning NINDS support for the traineeship of each of these faculty members will be added to these cards. This file, combined with the computerized file of neuroscientists now at the BIS in UCLA gives us a fairly good control of most of the manpower in research and teaching in the neurosciences. The practitioners of neurology, neurosurgery, and otolaryngology are all registered in the specialty directories. This is updated as new material which is received from the medical schools.

There have been many statistical compilations relating to brainpower in the clinical and nonclinical neurosciences. These were generated to assist the Extramural Program in its training program evaluation and to assist the director of the NINDS in his presentations about training programs.

Special Reports

There are always a number of special reports generated each year for a variety of reasons. Some are annual requests from DHEW or other parts of the government, some are generated by program needs and some are updating of old reports. The numbers of these reports has been markedly reduced because of the lack of professionally qualified personnel to write them. The most important reports of FY 1971 are:

Report on Neuromuscular Diseases, P.A. Report No. 31

This report includes a review of new research in neuromuscular diseases carried out during the year by Federal and Non-Federal agencies, especially those projects supported by the NINDS. It also includes a summary of the aims and status of each research project.

New Principal Investigators, FY 1970, P.A. Report No. 32

This report examined the age, sex, training and other characteristics of the investigators who received NINDS support for the first time in FY 1970. These characteristics were compared and contrasted with the new investigators of 1967 and 1957.

Federally Supported Agencies With Programs in Neurological and Sensory Disorders, P.A. Report No. 33

This document identifies governmental agencies other than those of the National Institutes of Health which have significant programs in the field of neurological and sensory diseases. It includes the sources through which one may obtain further information about an agency's program and lists that agency's specific projects.

The Aging Brain

This report identifies research projects supported by NINDS and other agencies on the physiological, chemical, cellular and morphological changes in the aging brain.

Accident (Brain and Spinal Cord Injuries), 1970-71

This is a review of research on the prevention, treatment and cure of neurological disorders caused by accidents, especially those concerning the head and spinal cord.

MR Research Supported by NINDS

This is the yearly updating of the NINDS Research Grants submitted to the Secretary's Committee on Mental Retardation of DHEW for the publication of "Mental Retardation Grants, Fiscal Year 1970."

International Activities Report

A report of NINDS international activities by coded program areas supported during FY 1970 was prepared for the Fogarty

International Center. This report included research grants, training grants, foreign research contracts, U.S. fellows and trainees abroad, visiting scientists, foreign working assignments, training of foreign nationals under domestic training grants, international conferences, and special foreign currency programs (PL-480).

This report was incorporated with an inventory (prepared for FIC also) of international activities of NINDS intramural scientists and foreign guest workers in a notebook for ready reference and updating as required.

Visiting Scientists

Program analysis has always tried to recruit visiting scientists to produce special reviews and surveys for the NINDS on subjects of special interest.

This past year Dr. David B. Shurtleff, Professor of Pediatrics at the University of Washington, Seattle, spent part of his sabbatical year studying the problem of congenital anomalies of the newborn and what is being done about them. This has been written up as Program Analysis No. 34 "National Support for Programs with Interest in Congenital Malformation of the Central Nervous System."

Workshops

The workshop activity has diminished considerably in the past year because of the decreasing budget. One workshop was held in connection with the Battelle Memorial Institute at Seattle on the status of acoustical holography as a diagnostic tool. The participants were physicists, engineers, neurologists, neurosurgeons and radiologists who met for one day at the Battelle Research Center in Seattle. The proceedings of this workshop are to be published first in summary form and later in some detail.

Neurological Information Network

This past year has seen much progress and development along with a few structural changes in the Neurological Information Network. Users of the Network's products and services are overwhelmingly enthusiastic; the current awareness bulletins, reports and reviews continue to receive national and international acceptance. Network activities now have, as originally planned, a greater emphasis on science-dependent products such as state-of-the-art reviews and syntheses of science information. There has thus been corresponding de-emphasis of bibliographic products made possible through the improved operation and service from MEDLARS.

The two centers in the Neurological Information Network, the Brain Information Service (BIS) at UCLA and the Information Center for Hearing, Speech, and Disorders of Human Communication (HSDHC) at Johns Hopkins, have maintained approximately the same level of activity as that of the preceeding year. A measure of their service to the NINDS supported scientists is the degree to which they are used by NINDS grantees. Out of 1,386 grantees listed in the

December 1970, Data Book, the Center at Johns Hopkins has answered 248 information requests from 186 grantees and 40 requests from 28 NINDS staff members. Most of these requests from grantees were from the 178 who are specifically working on problems of hearing and speech and language, who along with some 120 odd other grantees working on problems of the special senses are on their general mailing list.

Three hundred and fourteen NINDS grantees are among the 1200 scientists who receive current products from BIS, UCLA weekly or biweekly. Of the grantees on the HSDHC and BIS active lists only 29 are on both lists.

The Information Center for Hearing, Speech, and Disorders of Human Communication has continued the publication of Communication Disorders, changing from bimonthly to monthly publication and changing its title to Human Communications and Its Disorders. The center is trying to develop ways to provide this alerting service with the assistance of the National Library of Medicine's MEDLARS system. Part II of the guide Information Sources in Hearing, Speech and Communication Disorders has been published. The conference report on the Neuroanatomy of the Auditory System, held in May, 1970, is to be published in the Archives of Otolaryngology. A conference on the Physiology of the Auditory System is scheduled for June, 1971. Reviews completed this year are on hearing problems related to virus infections, and "The Relationship Between Surgical Correction of Tongue-Tie and Speech Defects", "Hereditary Deafness with External-Ear Abnormalities", and "Speech and the Dentofacial Complex: the State-of-the-Art". A 146 page bibliography produced for the Armed Forces Institute of Pathology seminar on the diseases of the middle ear, in addition to being distributed to the attendees, was published as a supplement to the Annal of Otology, Rhinology and Laryngology.

In preparation are: additional workshops on Neuropathology of the Auditory System and Traumatic Effect upon Hearing from Noise Exposure, an index handbook on Ototoxic Drugs, and a critical review of "Efficacy of Treatment Procedures in Reading Problems".

The Brain Information Service at UCLA maintained its high level of productivity during the past year. The updated review, The Dorsal Column System of the Spinal Cord was updated a second and third time and is being reprinted in its latest version. The biweekly alerting bulletin Biogenic Amines has continued to carry short reviews by Dr. Diana Johnson. Meeting reports are prepared by Dr. Johnson one month to six weeks after the meetings take place. These are published by BIS and also often appear in journals. For example, the meeting reports on International Symposium on Steriods, Gonadotrophins and Reproduction, and on Biology of Fertilization and Implantation were published by BIS appeared also in December, 1970 and January, 1971 issues of Contraception.

A workshop on neuronal models was held August, 1970 and a report is in preparation. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects has been so much in demand that it has been reprinted. The manual "Standardized Terminology, Techniques and Criteria for Scoring of Sleep-Wake Status of Full Term Infants" will be available in June, 1971. Dr. Doris Dunn has initiated "abstracts in depth" for inclusion with the BIS publication Neuroendocrine Control Mechanism: Hypothalamic-Pituitary-Gonadal System. A new alerting bulletin on memory and learning has been started and is published semimonthly. KWIC indices to EEG and allied literature, 1966-1969, is being published to cover the period between Dr. Bickford's KWIC Index of EEG Literature and the current BIS "KWIC" published annually.

Program Analysis staff has assisted the Special Projects Branch in developing and testing an on-line retrieval system for Epilepsy Abstracts. The data base is accessible for on-line queries and response at the Mead Data Central computer in Virginia. A study of the effectiveness and utility of the system is underway with the consultative assistance of Dr. F. W. Lancaster of the University of Illinois, School of Library Science. The results of the test may provide a basis for considering an expanded system to cover a wider area of neurology.

The Neurosurgical Biblio-Index is a collaborative venture of the Journal of Neurosurgery, NINDS and the NLM to utilize MEDLARS to produce a recurrent bibliography for neurosurgeons. A special survey is being conducted in an attempt to assess its value and the value of recurring bibliographies in general. This is especially important as recurring bibliographies are a common product of both the Network and the NLM, and the result may well affect the activities of both.

Parkinson's Disease and Related Disorders is a monthly alerting bulletin put out by the Office of Program Analysis as a continuation of that started by the former Parkinson's Information Center. It has a circulation of about 1200. The OMB has put a limit of 1500 for free circulation, and when it reaches this, it will be sold by GPO at $2.50 per year plus $.75 for foreign subscription.

The Cerebrovascular Disease Alerting Service, providing monthly bibliographical listings and abstracts of material from about 145 journals through a contract with the Mayo Foundation of Rochester continues to receive the commendations of its audience. The facilities at the Mayo Foundation have allowed distribution to only a small group, but the contract for next year calls for publishing the abstracts in the journal Stroke to achieve wider distribution.

THE NINDS FOREIGN CREDIT CURRENCY AWARD (PL-480)

The Foreign Credit Currency Award, or PL-480 program, is a program of research supported by United States owned foreign currencies which are not convertible to dollars. It is authorized by the Agricultural Trade Development and Assistance Act of 1954, Public Law 480, Sub-section 104 (K), which permits the use of foreign currencies, "to conduct and support scientific activities overseas including programs and projects of scientific cooperation between the United States and other countries, such as coordinated research against diseases common to all of mankind that are unique to individual regions of the globe and to promote and support programs of medical and scientific research." The funds are available in Burma, Ceylon, Guinea, Israel, Pakistan, Poland, Tunisia, UAR (Egypt), Yugoslavia, Indonesia, and India, but this list is subject to changes. The money in Israel is almost exhausted; and unless some new negotiations are concluded between the United States Government and the Government of Israel, this program will be phased out in Israel in the next two years. At the present time, no new projects are being accepted for funding under this program in Israel and the committed years will be supported in old projects only as long as the present funds last.

Each of these projects must have a U.S. scientist who serves as "U.S. project officer" while the foreign scientist is designated as the principal investigator. The role of the U.S. project officer may vary from that of collaborator actively participating in the research, to that of an informal sponsor.

In FY 71 the allotment of funds for the PL-480 program was separated from the dollar budgets of the Institutes. This has resulted in a considerable expansion of the program and makes for a clearer distinction between the use of these funds and the foreign dollar expenditures. Only U.S. owned foreign currencies are used in this program, and any needs for dollars must be met from other sources.

At the start of FY 71 the NINDS was supporting sixteen projects at $510,000: one project in UAR (Egypt) in the amount of $24,000; four projects in India at $41,000; four projects in Israel at $155,000; five projects in Poland at $192,000; and two projects in Yugoslavia amounting to $98,000.

The substance of these active research projects includes both the non-clinical sciences and clinical studies. Three studies are basically neurophysiological in nature, three are biochemical with some clinical correlates; two are immunological; three are epidemiological studies, two on cerebrovascular diseases, and one on multiple sclerosis; one is related to the NINDS perinatal research project; one is on muscle disease; and three are on brain tumors and the biology of glia.

Because of the confusing situation resulting from the lumping of PL-480 funds with the institutes' dollar budgets and the use of PL-480 cuts to save dollars prior to FY 71, a backlog of 22 approved projects in the amount of $398,385 accummulated. The substantial increases in the PL-480 funds will allow the payment of most of these projects except those in Israel. This will bring the NINDS total at the close of FY 71 to 33 projects at about $800,000.

The scientific areas of the backlog projects are: two in the chemistry of neurotoxic natural substances; two on the chemistry and functions of acetylcholinesterasea; one on biogenic amine metabolism; two on the biochemistry of brain lipids; one on immunochemistry of the brain; one on the vestibular system; one on histogenesis of the human fore brain; one on nutrition and human brain development; and one on head injury.

The increase in funds has stimulated the development of other new projects whenever suitable laboratories and scientists are identified. The development of the new projects is a slow process partly because of general communication difficulties and partly because most of the foreign scientists do not understand the U.S. requirements for detail on project applications. This second difficulty often necessitates a visit by the U.S. project officer or the foreign scientist visiting the U.S. in order to jointly work out an adequate proposal.

The PL-480 program had its beginning about 1963 and the accomplishments of this program have been real. As this is the first time the NINDS PL-480 program has been reviewed for the Annual Report this review of accomplishments will cover more than just FY 71.

Cerebrovascular disease and stroke are major neurological problems in the world. The NINDS is supporting research in Japan and India on the epidemiology of stroke in order to clarify reports of different incidence and prevalence of the cerebrovascular disease problems throughout the world. The Indian projects are supported through PL-480 funds.

The arterial degenerative disease process may be different in the tropics as is evidenced in India where a process appears similar to atherosclerosis but without the deposition of cholesterol. Also, there are many strokes in the younger age groups. Investigations are continuing to see if dietary or other environmental or casual factors might be identified which accounts for these differences.

Another contribution in India (P.M. Dalal) is the discovery that there is a clot dissolving factor which comes out of the blood vessel wall as a consequence of the lowered oxygen availability in the blood vessel wall then blood flow stops. This is of considerable importance in understanding of the problems of arterial thrombosis and might be a lead to effective therapy.

Muscular disorders are a group of devastating diseases affecting the neuromuscular system. There has been an extremely productive program between the Medical Neurology Branch, NINDS, and Dr. I. Hausmanowa-Petrusewicz in the Department of Neurology of the Medical Academy of Warsaw, Poland. This laboratory has developed into one of the most productive laboratories in Poland and is continually producing new information at all levels, basic and clinical, about this group of diseases. They have recently produced an atlas which describes the main pathological features of muscle diseases and is a definitive work on the subject.

Sclerosing disorders, including multiple sclerosis, are a major problem because they are of unknown etiology and of long duration. The study of brain lipids is of particular importance, but research was difficult because of lack of synthetic lipids which could be radio tagged to study their metabolism. At the Weizmann Institute in Israel under the direction of Dr. Shapiro, an unusually gifted synthetic lipid chemist, the synthesis of many of the brain lipids and their substructure molecules has been carried out. This work is almost unique in the world and these synthetic products have made possible much of the research in this area. These synthetic substances are also used to study the diseases of inborn errors of metabolism as well as multiple sclerosis. This has been of considerable help to the U.S. project officer, Dr. Roscoe Brady, of the NINDS in his now well known work in Tay-Sachs disease.

In the area of inborn errors of metabolism, an important contribution has been made by Dr. B.K. Bachhawat in Vellore, India, working on a PL-480 grant with Dr. James Austin in Denver. These investigators have succeeded in isolating the specifically missing enzymes of metabolic deficiency diseases, globoid metachromatic leukodystrophy. This research interaction has taken place through exchange of materials and technological skills between the group in Denver and the group in India. It represents a real discovery of the basic cause of a disease which results in mental retardation. Such discoveries are the first step toward therapy.

The two projects on the biology of brain tumors which are supported in Poland through the PL-480 program have added significantly to our knowledge about the biology and histochemistry of brain tumors. This data is important in the development of new chemotherapeutic drugs and for developing biochemical methods of assessing tumor growth.

The study of the naturally occuring neurotoxins in animals and plants is the subject of three projects. One on the venoms and anti-sera of the poisonous snakes of Africa is being carried out by Dr. A. Mohamed in Cairo, Egypt, and Dr. R.L. Irwin of the Medical Neurology Branch, NINDS. Details of this work are covered in Dr. Irwin's report. The other two projects, in India, are on snake venoms and plant neurotoxins. These substances are of great interest because of their devastating effect on the nervous system function. Knowledge of their mode of action may be of importance in understanding of the CNS. Some of these poisons also are useful as physiological tools. In addition to studying their mode of action, anti-sera to these poisons are being developed which are of immense therapeutic value.

Studies on the fundamental processes of alimentary reception mechanisms in different nutritional states are being carried out by Dr. K.M. Sharma in collaboration with Dr. K. Frank. Dr. Frank and his associates have spent considerable time in India helping to get this project properly underway.

As a result it will be not only productive of solid data but will be equally important by developing a good neurophysiological research laboratory in a country where few exist.

An outstanding example of cooperative effort is the International Brain Research Laboratory at Kotor which is a joint project between the Brain Research Institute at UCLA and the University of Belgrade under the direction of Ljubisa Rakic. This laboratory is a very good example of a foreign country sharing the cost of the project with the U.S.A. The input from Yugoslavia has not only been as much in monetary investment but, when the relative investment is compared to the available resources of each country, it has been enormous. The International Brain Research Laboratory located at Kotor is part of the new multidisciplinary faculty of the University of Belgrade. The research carried on retinal and visual physiology, neurophysiology of various kinds, and immunology of the nervous system. The biological rhythms of the nervous system is another area of great interest. There is continual interaction with American scientists on these projects and there is a visiting scientist program whereby scientists from the United States can regularly visit. There have been some 27 visiting scientists from different countries in the past year. Kotor is becoming a popular site for international meetings on many subjects related to the neurosciences.

While substantial progress has been made in developing the laboratory at Kotor from scratch, it needs strengthening of the full time faculty of senior scientists who devote their full time to work at Kotor without having teaching obligations at the University in Belgrade. This need stems from the scarcity of really first class scientists in Yugoslavia, and plans are being made to alleviate this in part by protracted visits from established scientists from elsewhere.

In addition to the research projects cited, the NINDS PL-480 program has supported visiting scientists who come to the Bethesda campus to work with our intramural scientists. These scientists bring ideas and new developments from other sections of the world and introduce them into the American scientific community. Many of these are small contributions but they are solid bricks in the foundation of scientific knowledge upon which all our therapeutic efforts are eventually based. This interaction of scientists has resulted in creating an image of NINDS and the NIH that is considerably larger throughout the world than its image in the United States.

Egypt

Number	Title	Principal Investigator	Project Officer
03-006-1	To study the Venoms of the Poisonous Animals (Snakes and Scorpions) within Egypt for the Development of New and Improved Antisera	Dr. Ahmed M. Mohamed Physiology Dept. Faculty of Medicine Ain Shams Univ. Cairo, Egypt, U.A.R.	Dr. Richard L. Irwin Head, Section on Applied Pharmacology Med. Neurology Br. NINDS

Current Obligation
Local Currency: 75,264 U.S. Dollars: 135,466 Status: Active

Period: 5/24/67-4/30/72

India

Number	Title	Principal Investigator	Project Officer
01-040-1	Polypeptide Constituents of Snake (naja naja)	Dr. L. K. Ramachandran Osmania University Hyderabad, India	Dr. H. Fraenkel-Conrat Univ. of Calif. Berkeley, Calif.

Current Obligation
Local Currency: 481,220 U.S. Dollars: 63,318 Status: money obligated, final negotiations in progress
Period: 71-74

01-034-1	Neurotoxins from Plant Materials	Dr. G. Padmanaban Asst. Prof., Dept. of Science, Indian Inst. of Science Bangalore, India	Dr. Frank M. Strong Dept of Biochem. College of Agriculture Madison, Wis.

Current Obligation
Local Currency: 186,673 U.S. Dollars: $24,562 Status: money obligated, final negotiations in progress
Period: 71-74

01-041-1	Effect of Protein Malnutrition on Brain Metabolism	Dr. Saroj Mehta Senior Lecturer in Postgraduate Med. Institute Chandigarh, India	Dr. John Menkes Department of Pediatrics, UCLA Sch. of Medicine Los Angeles, California

Current Obligation
Local Currency: 343,335 U.S. Dollars: $45,175 Status: money obligated, final negotiations in progress

01-011-1	Collaborative Neurologic Investigations: Boston/Bombay	Dr. Darah K. Dastur Neuropathology Unit ICMR Bombay, India	Dr. Raymond D. Adams Mass. General Hospital Boston, Mass.

Current Obligation
Local Currency: 289,252 U.S. Dollars: $38,059 Status: Active

India (cont'd)

Number	Title	Principal Investigator	Project Officer
01-015-1	Role of Central & Peripheral Neural Mechanisms in the Regulation of Feeding Behavior	Dr. Kamal N. Sharma St. Johns Medical College St. Mary's Town Bangalore 5, India	Dr. Karl Frank Laboratory of Neural Control NINDS/NIH

Current Obligation
Local Currency: 117,680 U.S. Dollars: $15,484 Status: Active

Period: 3/15/69-3/14/72

01-024-1	Collaborative Neurologic Studies: Denver/Vellore	Dr. Bimal K. Bachhawat Neurochemistry Lab. Christian Medical College & Hospital Vellore, South India	Dr. James H.Austin Prof. of Neurology Univ. of Colo. Medical Center Denver, Colo.

Current Obligation
Local Currency: 390,000 U.S. Dollars: $51,316 Status: Active

Period 4/1/69-3/31/72

01-016-1	Collaborative Neurologic Investigations of Cerebrovascular Disorders	Dr. Praful Dalal Prof. of Medicine and Neurology, Dept. of Medicine, B.Y.L. Nair Charitable Hosp. Bombay, India	Dr. Raymond D. Admas, Chief Neurology Service Mass. Gen. Hos. Boston, Mass

Current Obligation
Local Currency: 158,462 U.S. Dollars: $21,138 Status: Active

Period: 1/28/69-6/30/72

India (cont'd)

Number	Title	Principal Investigator	Project Officer
01-099-1	Investigation into the Causes and Treatment of Non-Traumatic Paraplegia with Particular Reference to Arteriovenous Malformations of the Spinal Cord	Dr. R.N. Chatterjee Prof. of Neurosurgery Institute of Post Graduate Medical Education and Research Calcutta, India	Dr. Ayub K. Ommaya NIH/NINDS

Current Obligation
Local Currency: 632,530 U.S. Dollars: 83,220 Status: Approved by Technical Merit Review and Inst. Review

Israel

Number	Title	Principal Investigator	Project Officer
06-043-1	Synthetic Sialic-Acid Containing Oligosaccharides	Dr. Harold M. Flowers Weizmann Inst. of Science P. O. Box 26 Rehovoth, Israel	Dr. Roger W. Jeanloz Mass. General Hospital Boston, Mass

Current Obligation
Local Currency: 147,095 U.S. Dollars: 42,027 Status: Pending probably will not be funded

06-095-1	Single Unit Study of Acquired Functional Connections between Sensory and Motor Systems	Dr. Moshe Abeles The Hebrew Univ. Jerusalem, Israel	Dr. Moise Goldstein Johns Hopkins University Baltimore, Md.

Current Obligation
Local Currency: 288,625 U.S. Dollars: 82,464 Status: Pending probably will not be funded

06-028-1	Molecular Biology and Immunology of Trachoma Agents	Dr. Yechiel Becker Hebrew University-Hadassah Med. School Jerusalem, Israel	Dr. Ernest Jawetz San Francisco Med. School San Francisco, California

Current Obligation
Local Currency: 178,100 U.S. Dollars: 50,886 Status: Active

Period: 4/1/72-3/1/73

Israel (cont'd)

Number	Title	Principal Investigator	Project Officer
06-008-1	Neural and Endocrine Factors Regulating Hypothalamic Activity	Dr. Shaul Feldman Director Lab. of Neurophysiology Hadassah Univ. Hosp. Jerusalem, Israel	Dr. Julian M. Davidson Dept. of Physiology Stanford Univ. Stanford, Calif.

Current Obligation
Local Currency: 84,880 U.S. Dollars: 72,045 Status: Active

Period: 7/1/70-6/30/73

06-011-1	Epidemiologic Study of Multiple Sclerosis in Israel	Dr. Uri Leibowitz Hadassah Med. Org. P.O. Box 499 Jerusaleum, Israel	Dr. Milton Alter, Chief Neurology Service Veterans Admin. Hospital 54th St. & 48th Ave. So. Minneapolis, Minn. 55417

Current Obligation
Local Currency: U.S. Dollars: Status: Probably will not be funded

Period: 6/29/71 (expired)

06-015-1	Synthesis of Sphingolipids for Use in Determining the Metabolic Aberrations in Hereditary Sphingolipidoses	Dr. David Shapiro Prof. of Organic Chemistry Weizmann Inst. of Science Rehovoth, Israel	Dr. Roscoe O. Brady, Jr. Head, Sec. on Lipid Chemistry Lab. of Neurochemistry, NINDS

Current Obligation
Local Currency: 165,000 U.S. Dollars: 47,143 Status: Active

Period: 8/10/70-8/9/72

Poland

Number	Title	Principal Investigator	Project Officer
05-004-1	Biology & Pathology of the Glial Tissue	Dr. Miroslaw Mossakowski Dept. of Neuropathology Polish Academy of Sciences Warsaw, Poland	Dr. Igor Klatzo, Head, Section on Clinical Neuropathology Surgical Neurology Br. NINDS

Current Obligation
Local Currency: 3,469,900 U.S. Dollars: 144,204 Status:

Period: 6/1/68-5/31/71

05-001-01	Preconceptual Causes of Defects in Children	Dr. Ireneusz Roszkowski Dept. of Gynecoy & Obstetrics Warsaw Academy of Med. Warsaw, Poland	Mr. William Weiss, NINDS Co-Project Officer Dr. Heinz Berendes NINDS

Current Obligation
Local Currency: 4,199,840 U.S. Dollars: 174,993 Status: Active

Period: 8/1/68-7/31/71

05-002-01	Clinical and Laboratory Characteristics of Muscle Disease	Dr. Irena Hausmanowa-Petrusewicz Dept. of Neurology Medical Academy of Warsaw Oczki 6 Warsaw, Poland	Dr. W. King Engle Chief, Medical Neurology Br. NINDS

Current Obligation
Local Currency: 2,167,892 U.S. Dollars: 90,329 Status: Active

Period: 6/1/71-5/31/73

Poland (cont'd)

Number	Title	Principal Investigator	Project Officer
05-013-1	Biology and Histochemistry of Gliomas	Dr. Andrzej Gluszcz Pathology Institute Medical Academy Lodz, Poland	Dr. Edgar A. Bering, Jr., Special Asst. for Program Analysis NINDS

Current Obligation
Local Currency: 1,567,800 U.S. Dollars: 65,325 Status: Active

Period: 8/1/68-7/31/71

05-027-1	Biochemical and Histochemical Aspects of Myelination & Demyelination in the Central Nervous System	Dr. Mieczyslaw Wender Medical Academy Poznan, Poland	Dr. Igor Klatzo NIH/NINDS

Current Obligation
Local Currency: 4,810,716 U.S. Dollars: 200,447 Status: money obligated, final negotiations in progress

05-028-1	Anoxemic Damage of the Immature Nervous System	Asso. Prof. Maria Dambska The Polish Academy of Sciences Dept. of Neuropathology ul. Dworkowa 3, Warsaw	Dr. Ronald Myers NIH/NINDS

Current Obligation
Local Currency: 1,177,990 U.S. Dollars: 49,082 Status: money obligated, final negotiations in progress

Yugoslavia

Number	Title	Principal Investigator	Project Officer
02-003-1	Neural Correlates of Behavior	Dr. Radoslav K. Andjus Director Institute for Biology Academy of Sciences Belgrade, Yugoslavia	Dr. John D. French Director Brain Research Institute Univ. of Calif. Los Angeles, Calif.

Current Obligation
Local Currency: 1,552,749 U.S. Dollars: 124,220 Status: Active

Period: 10/1/70-9/30/72

02-020-1	The Function and Biosynthesis of Ribosomes	Dr. Valdimir R. Glisin Institute of Biology Univ. of Belgrade Belgrade, Yugoslavia	Dr. Paul Doty Harvard Univ. Dept. of Chemistry Cambridge, Mass.

Current Obligation
Local Currency: 917,477 U.S. Dollars: 73,398 Status: Active

Period: 10/15/70-10/14/72

02-006-1	Histogenesis of the Forebrain in Human Embryos	Dr. V. Sulovic Assoc. Prof. of Gynecology Dept. of Neurology Belgrade Univ. Med. School Belgrade, Yugoslavia	Dr. Richard L. Sidman Dept. Neurology and Neuropathology Harvard Med. Sch. Cambridge, Mass.

Current Obligation
Local Currency: 516,875 U.S. Dollars: 41,350

Yugoslavia (cont'd)

Number	Title	Principal Investigator	Project Officer
02-008-1	Function of Cholinesterases in Normal and Pathological Conditions	Dr. Andrej O. Zupancic Univ. of Ljubljana Ljubljana, Yugoslavia	Dr. Melvin Yahr Columbia Univ. New York, N.Y.

Current Obligation
Local Currency: 1,335,400 U.S. Dollars: 106,832 Status: money obligated final negotiations in progress

02-015-1	Metabolism, Release and Uptake of 5-hydroxytryptamine "In Vivo" and "In Vitro"	Dr. Zlatko Supek Inst. Ruder Boskovic Zagreb, Yugoslavia	Dr. R. Merlin Bumpus Cleveland Clinic Cleveland, Ohio

Current Obligation
Local Currency: 848,750 U.S. Dollars: 67,900 Status: money obligated, final negotiations in progress

02-023-1	Vestibular Influence on Cerebral Activity	Dr. Bosko Barac Univ. of Zagreb School of Medicine Zagreb, Yugoslavia	Dr. Gilbert H. Glaser Prof. of Neurology School of Med. New Haven, Conn.

Current Obligation
Local Currency: 692,623 U.S. Dollars: 55,000 Status: money obligated, final negotiations in progress

Yugoslavia (cont'd)

Number	Title	Principal Investigator	Project Officer
02-025-1	Investigation of Neuropathology & Pathogenetical Mechanisms of Closed Brain Injury of the Acceleration-Deceleration Type	Dr. Nenad Grcevic Prof. of Neuropathology Faculty of Medicine University Hospital Zagreb, Yugoslavia	Dr. Keffin Penry NIH/NINDS
Current Obligation Local Currency: 349,900	U.S. Dollars: 27,992	Status:	money obligated, final negotiations in progress
02-027-1	The Molecular Weight of Acetylocholinesterase	Dr. Milos R. Pavlic Univ. of Ljubljana Ljubljana, Yugoslavia	Dr. Irwin B. Wilson Univ. of Colo. Boulder, Colo.
Current Obligation Local Currency: 349,750	U.S. Dollars: 27,980	Status:	money obligated, final negotiations in progress
02-032-1	Sialidase Action on Complex Molecular Aggregates	Dr. Vaskresenija Lipovac Univ. of Zabreb Zagreb, Yugoslavia	Dr. Abraham Rosenberg Dept. of Biological Chemistry Penn. State Univ. Hershey, Pa.
Current Obligation Local Currency: 683,117	U.S. Dollars: 54,645	Status:	money obligated, final negotiations in progress

Yugoslavia (cont'd)

Number	Title	Principal Investigator	Project Officer
02-041-1	Influence of Calcium and Magnesium Ions or Release of Acetylcholine and Possible Transmitter Function of Biogenic Amines in the Brain	Dr. Mirjana Randic Lab. of Neuro-pharmacology Inst. "Ruder Boskovic" Zagreb, Yugoslavia	Dr. Choh-luh-Li NIH/NINDS

Current Obligation
Local Currency: 152,600 U.S. Dollars: 76,840 Status:

Number	Title	Principal Investigator	Project Officer
02-FIC 133	Nutrition as a Factor of Perinatal Injuries	Prof. Jovan Cekic Director Inst. for Health Protection The Socialist Republic of Serbia 12 Dr. Subotica 5 Belgrade, Yugoslavia	Dr. Berendes/ Dr. Weiss NIH/NINDS

Current Obligation
Local Currency: 2,167,870 U.S. Dollars: 144,524 Status: Pending review

PROJECT REPORT

Serial No.: NDS(I)-71 LNP/NI 1934
1. Laboratory of Neurophysiology
2. Section on Neuronal Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electroencephalograph Calibrator for Use in Determining Electrocerebral Silence

Previous Serial Number: None

Principal Investigator: Edward F. MacNichol, Jr.

Other Investigators: None in section

Cooperating Units: Technical Development Section NIMH/NINDS
Electroencephalography Branch
Office of Biometry
Office of Information

Man Years:

Total: Less than 0.1
Professional: Less than 0.1
Other: 0

Project Description:

Objectives: 1) The development of a method and device for use in cases of suspected cerebral death to evaluate the condition of the entire EEG system including the application of the electrodes to the patient in order to assure that electrical activity larger than a pre-assigned value (2 microvolts) would be recorded if present. 2) The evaluation of the method and device. 3) The construction of an exhibit for demonstrating a working model at the American Neurological Association's annual meeting on June 14, 15, and 16, 1971.

Methods Employed: Absence of a recognizable trace that indicates electrocerebral activity on the EEG record is a negative criterion that can be caused by factors other than electrocerebral silence such as equipment malfunction, or a high ambient level of electrical interference which would mask the EEG signal. Therefore an objective method of determining whether or not electrocerebral activity would be recorded if present is badly needed. Furthermore, electrocerebral silence is an important criterion upon which a decision to terminate resuscitative measures is based. Medico legal questions are involved, particularly when the patient is a potential donor of vital organs.

An initial attempt to furnish a test signal that would check the entire system was the development of a constant current square wave generator having a frequency of about 1 Hz that would inject a signal between chin and vertex of the patient. A pilot model of this device (Injector) was built by the Technical Development Section and tested on a series of patients in the Electroencephalography Branch. The ratios of output potential in various lead pairs to input current was measured.

Analysis of the records by the Office of Biometry indicate a large variation in output between different leads on the same patient and within identical leads on different patients. Thus the presence of a tracing on a given channel shows that the system is working and that the electrodes are functionally connected to the patient, but does not provide a known calibrating signal to compare with the amplitude of the EEG A commercial version of this device developed independently by the Grass Instrument Company, is being made available to investigators for evaluation.

In order to provide a calibrating signal a closed transformer core with a primary winding is used. One of each pair of EEG leads is threaded through the aperture in the core and a suitable waveform applied to the primary to induce a 1 Hz square wave of known amplitude in each single turn secondary. Since the half period of the 1 Hz square wave is much below the L/R time constant of the primary the transformer takes the time derivative of the input waveform, which is therefore a symmetrical ramp function. This is generated by a square wave input which is compared with the output of a feedback winding on the core.

This device (Inductor) was built and tested. It gives a satisfactory calibrating signal and tests the entire system including the leads. It does not indicate whether the leads are in good contact with the patient's scalp.

To determine whether the leads are connected to the patient's scalp and to provide a known calibrating signal both devices are needed. A combined device has been constructed by the Technical Development Section. It is currently being evaluated. The induced potential and injected current waveforms are generated in sequence at programmed intervals so that they are automatically displayed on the EEG record.

A working model of the combined device using a doll with conductive material inside its head instead of a patient is being prepared for exhibit by the Office of Information.

Eight other prototypes are being constructed by the Technical Development Section.

Significance to Biomedical Research and the Program of the Institute:

The NANDS Council has urged the Institute to undertake a study of cerebral death. This study is entering its pilot phase in six collaborating institutions. It is hoped that the prototype units can assist this study by providing indications of the functional state of the EEG systems to those who will evaluate the records.

Honors and Awards: None

Publications: Paper on "Positive Criteria for the Determination of Electrocerebral Silence" to be read by title at the 96th Annual Meeting of the American Neurological Association will be published in the *Proceedings*.

Office of Biometry

The Office of Biometry, NINDS, is a Branch within the Office of the Director, NINDS, and operates under the direction of the Deputy Director of the Institute. The primary objectives of the Office of Biometry are to provide all organizations of NINDS with a central resource for service in statistics, mathematics, data processing and computer technology. The services range from brief consultations to complete execution of major projects. A secondary aim is to provide consultation and service to other organizations undertaking research in the area of NINDS interest.

The Office of Biometry consists of the Office of the Chief and three Sections. The Section on Mathematical Statistics provides statistical, mathematical, and computational services in the design, analysis, and evaluation of research experiments. The Section on Applied Statistics provides statistical analysis and computational service in the design, analysis and evaluation of collaborative prospective investigations and other analytical studies. The Section on Systems Design and Data Processing has responsibility for systems analysis and data processing as well as for the utilization of machines and equipment related to computers or computer output.

The review of projects and services performed by the Office of Biometry is listed by Branch or Laboratory. This is followed by description of Research, Future Plans, Miscellaneous Activities and Publications.

OFFICE OF THE DIRECTOR, NINDS

1. Advice on data processing. The Office of Biometry provides advice and information on present and future data processing needs of NINDS.

2. EEG Checker study. Statistical services were provided in two phases of the EEG Checker project.

   PHASE I. A statistical analysis was carried out on data provided by the EEG Branch for a study of a device (EEG Checker) for checking soundness of connections to the patient and the operation of each channel of EEG machines which are being used in the determination of cerebral death. EEG recordings from 16 test subjects were quantified and analyzed with ANOVA (analysis of variance) methods to determine sources of variation in EEG response to checker input signals. Large variations were observed among patients, test days, EEG pulses. In addition, correlation analysis revealed no marked relationship between responses and some human characteristics such as age, sex, head circumference, etc. Recommendations were made for experimental design and procedures to be used in a later phase study.

PHASE II. This study was aimed at deriving a suitable test criterion to check EEG lead connections. A pilot experiment was first run to obtain an optimum sensitivity of the EEG recordings. A highly automated computer data filing and updating system was devised, enabling successive analyses to be performed involving all the data received up to the time of analysis. The experimental work of the main study has not been completed due to the fact that a new checker, considered to be more efficient than the currently tested one, has been developed and further experiment may be extended to testing the new checker alone and comparing the two checkers. Statistical analysis was carried out on data available from 25 patients so far recorded, to reinvestigate various sources of variations in the EEG response. Results were compared with those obtained in the Phase I study. A status report was prepared and submitted to the Director. Discussion of the problems in deriving a test criterion for checking EEG lead connections was presented in the report.

3. Carotid occlusive disease and photoelectric plethysmography. A carotid occlusive disease screening method using supraorbital photoelectric plethysmography, was analyzed for false positives and false negatives. The plethysmography recordings were obtained on patients, 18 of whom were negative and 19 positive by the angiography test. Pulse wave recordings on the contra- and ipsi-lateral side of the supraorbital region of each patient were obtained and quantified. This included recordings before, during, and after thumb compression on the neck. Two criteria based on pulse amplitude or phase were computed for each patient to provide indication of ICA stenosis. These results were then checked with their respective angiography findings. Notably high rates for both false positives and false negatives of this method were observed. An analytical report was prepared and submitted to the investigators. Possible methods of improving the screening procedure and other discussions were also given.

4. Grouping EEG leads for calibration purposes. A service was provided in a project studying cerebral death. The basic problem is that, in order to use the magnetic induction calibrator to calibrate all EEG channels in the five electrode montages that are being planned in the cerebral death study without changing the way the leads are passing through the signal inductor core, the EEG leads must be divided into 3 separate groups so that no two leads in the same group are connected. The application of a principle of combinatorial analysis yielded 32 possible ways of such grouping. A report presenting the procedure and an example of application was submitted to the Director.

5. Plan for evaluating the effectiveness of epilepsy abstracts retrieval system. Consultation on a study design involving subjective evaluation of how effectively the EARS system can be used by specialists in neurology, when searching in a time-shared mode to extract research information on epilepsy. Suggestions were given for the improvement of the design, in order to increase the validity of the results.

6. The Chief of the Office of Biometry attends all meetings of the Perinatal Research Committee, which directs the research programs of the Collaborative Perinatal Study.

   The Chief is also a member of the Epidemiology and Statistical Analysis Committee, which is an advisory body to the Perinatal Research Committee. The Office of Biometry provides consultation to both committees.

7. General data processing support for the Office of the Director.

   (a) The Office of Biometry provided a quick retrieval reprint file for neurological references.

   (b) The Office of Biometry assisted the Office of Program Analysis by providing key punching specifications and data processing.

8. EEO Program. The Office of Biometry, as part of its efforts to improve equal employment opportunities of its staff, conducts active in-house training programs for personnel in the lower grade levels. Secretaries and statistical assistants are thereby encouraged to develop new skills, primarily in the programming area, which are greatly needed by OB. The Office of Biometry has offered to make its professionals available to the Institute for statistical and programming training of personnel in dead-end jobs. It is now participating in a program to develop computer accessed lists of NIH position vacancies, and match them to applicants.

COLLABORATIVE AND FIELD RESEARCH

OFFICE OF ASSOCIATE DIRECTOR

1. Measles and myxovirus antibodies study. Statistical analyses were carried out for a research paper entitled "Measles and other Myxovirus Antibodies in the Cerebrospinal Fluids of Patients with Multiple Sclerosis and Other Neurological Diseases." Comparisons among various kinds of antibody titers in the serum as well as the CSF were achieved by constructing confidence intervals for the usual product moment correlation coefficient and Kendall's τ, a distribution-free correlation measure. The results of five distinct measles antibody tests were statistically tested on an individual basis by means of a binomial-based test; the results were pooled and overall significance was found by using a permutation (randomization) test. Using distribution theory associated with the multinomial distribution, a nonparametric test for the comparison of two geometric means was constructed. This test was used to compare measles antibody levels in control and MS patients - in both CSF and serum, while controlling for sex. A few of the more important results: 1. the CSF frequency of measles antibody was significantly greater in the MS group than the control group and 2. for the sensitive mixed-hemadsorbing antibody test, the geometric mean antibody titer was also significantly higher in the MS than the control group.

2. Normative statistics for hematological and serum chemical measurements. Normative statistics were derived from hematological and serum chemical measurements from a "clinically normal" group of inoculated rhesus monkeys. These statistics will be used (if clinical methods fail) to diagnose the presence of illness in various body organs and thus will expedite quick treatment of sick animals. Computer programs have been developed to plot the data on normal probability scale. Appropriate statistics for the identification of outliers will be devised and appropriate computer programs will be developed.

3. Kuru-Laboratory Study. The Office of Biometry provided statistical analyses for laboratory studies of Kuru and control patients. Means, standard deviations, t-tests and ANOVA's were computed on complete blood counts, liver function, cerebrospinal fluid chemistry urine, etc. These analyses summarized the data and tested for differences amongst Kurus by stage, age and sex, and among controls by age and sex. A paper on this project, "Kuru-Laboratory Studies", will be submitted for publication.

4. Data processing of Kuru data. OB provided consultation on methods of setting up a punched-card file on verifying, sorting, listing, and processing previously punched cards on Kuru patient data sent to NINDS by collaborators in Australia.

5. Albutoin study. The Albutoin trials are completed, and all of the clinical data has been processed and put into computer storage. Preliminary analyses have been performed, and presented to the ad hoc committee. The blood level material is nearing completion at New Castle and, as soon as it is finished, this material will be incorporated with the clinical data to give more definitive results.

6. Clinical evaluation of carbamazepine. The Office of Biometry developed a research methodology from pilot phase to final clinical evaluation for the promising new drug Carbamazepine. The study is to follow the termination of the Albutoin project. Our experience with Albutoin on the New Castle State Hospital population was most helpful in setting up the guidelines for this new study.

7. Clonezepam in the treatment of absence petit mal epilepsy. The Office of Biometry reviewed the project and suggested changes in the experimental design. Through discussions with the investigators, possible biasing or confounding factors were recognized and, where possible, were eliminated or accounted for in the proposed analysis of the data.

8. Anticonvulsants in man. The Office of Biometry is preparing a paper which will be presented at the Symposium on pharmacology of antiepileptic drugs. The paper will concentrate not only on the use of sophisticated statistical methodology in the testing of anticonvulsants, but also on the more rudimentary problems which are often overlooked and often lead to experimental results of lesser quality.

9. Guidelines for testing anticonvulsant drugs in man. Guidelines for the testing of anticonvulsant drugs were presented to the NINDS ad hoc committee as a joint effort of the Office of Biometry and the Section on Epilepsy. The guidelines, with comments by the committee, were then forwarded to the FDA for further comment and final publication in the Federal Register.

   The guidelines provide a basis for sound experimental research in the epilepsies without stifling ingenuity in experimental design.

10. Antiepileptic drugs. The Office of Biometry thoroughly reviewed the material compiled by Dr. James J. Coatsworth in his comprehensive examination of the literature dealing with the testing of antiepileptic drugs. Comments and suggestions were incorporated in the author's final draft.

11. Primate maternal nutrition study. This study of caloric and protein restrictions in pregnancy and their effect on biological variables and biochemistry of newborn rhesus monkeys was proposed as a collaborative effort by NINDS, NICHD, and the Johns Hopkins University Department of Pediatrics. The proposal received a favorable scientific merit review by an ad hoc committee, by the NINDS Directorate, and by NICHD. Joint funding by NINDS and NICHD was agreed upon.

    A pilot study of 38 pregnant monkeys to test the adequacy of the experimental design, and particularly the four dietary regimens, is well underway. Regular consultation has been maintained among all the investigators concerned, and periodic reports cover all of the points of current interest. This office has established an experimental design suitable for evaluating the results of maternal diet deprivation in rhesus monkeys, and is participating in the statistical analysis.

12. General data processing support for C&FR.

    (a) The Office of Biometry constructed and maintained files and processed data for a study of sub-acute Pan-Encephalitis, and for a literature file on epilepsy and head injuries.

    (b) The Office of Biometry developed and maintained the Spike-Wave Paroxysm Cohort System and the Quick Plot System for use by non-programming personnel. These programs, which use the Wylbur time - sharing system and the Cal Comp plotter, produce tables and graphs.

PERINATAL RESEARCH BRANCH

1. The Collaborative Perinatal Project. The Collaborative Perinatal Project is a major program of NINDS. The Office of Biometry supports it with a significant part of its resources.

(a) The Office of Biometry has at least one participating member in each of the many task forces which have been organized to develop, analyze, and report major studies of the Collaborative Perinatal Project. Frequently the OB staff has important responsibilities in the studies proposed by the task forces. Consultations with the Perinatal Research Committee and membership in the Epidemiological Statistical Advisory Committee have already been mentioned as contributions to the Office of the Director.

(b) The Office of Biometry staff collaborates with staff members of the Perinatal Research Branch in various special studies, or responds to requests for statistical and/or data processing assistance.

(c) The Office of Biometry provides statistical and data processing services for studies originating in participating Collaborating Institutions as well as in other organizations with an interest in perinatal research.

(d) The Office of Biometry investigates statistical methodologies and data processing techniques intended to be applicable to a broad spectrum of studies of Collaborative Perinatal Project data.

The type of work effort falls into two broad areas: Statistics, and Data Systems and Data Processing. The first includes formulation of hypotheses, development of the study designs, utilization or development of appropriate statistical techniques, and interpretation of results. The second includes design of forms; coding of forms for computer storage; creation, maintenance, and updating of files; specification writing, programming, and data processing for analytical studies, construction of tables, and graphic presentations. Some of the significant efforts in the Collaborative Study are:

2. CMV antibody. The Office of Biometry provided statistical consultation and services in a prospective serological study of cytomegalorvirus antibody in patients with carcinoma in situ of the uterine cervix. Antibody titer data of 168 serum samples from 45 patients and 90 controls were obtained. The patients were divided into three groups based on the stage of disease during which they were collected. Comparisons of titer levels were made between patients and controls within each group for serological findings for CMV. Significance tests based on parametric and nonparametric approaches were employed to derive levels of significance for each comparison. Analytical results were submitted and discussed with the principal investigator. The final draft of the manuscript for publication was reviewed for statistical appropriateness.

3. Descriptive monographs on the Collaborative Perinatal Study. A very considerable effort of the Office of Biometry has been made to complete a book "The Collaborative Perinatal Study - The Women and Their Pregnancies" the first of a series of descriptive reports of this long-term, large-scale study. The major work effort in this FY has concentrated on the review of the tabulations and charts, checking

them repeatedly for errors and inconsistencies, and evaluating the need for combining sub-groups or providing additional sub-groupings for clarity. The review committee, with intensive participation by the Office of Biometry, met repeatedly, either as an entity or in smaller work groups, to review the material and to prepare the commentary for the manuscript. The manuscript went through a number of drafts to reach its final form and will soon undergo final committee review.

The first book provides the detailed obstetric report; the second, which is the pediatric report, "The First Year of Life", is well on the way to completion.

4. Four and Seven-Year Neurological and Psychological Task Force. Investigated the utility of different methods of step-wise regression, of alternative methods of discriminant analysis, and of a non-orthogonal four-way analysis of variance.

5. Drugs and Anesthesia Task Force. Participated in the development of a study of the impact of labor and delivery drugs on Apgar score and its association with pregnancy outcome. Tabulations and special data tapes were provided to an ESAC consultant for testing of a proposed multiple regression technique.

6. Physical Growth and Development Task Force. Computer programs have been developed and tested which will be used to create a large set of charts showing the deciles of all physical growth characteristics of the Collaborative Study children.

7. Prenatal factors as they affect neurologic and psychologic outcomes. In collaboration with the Section on Pediatric-Neurology, a proposal was prepared to analyze the effects of certain prenatal factors on the neurological and psychological outcomes in the children of the Collaborative Study, utilizing a 1-1 matched control design.

8. Prepregnant weight and weight gain. The Office of Biometry has been invited to write a chapter on the "Physical Characteristics of the Gravida and their Association with Birthweight and Perinatal Death" for *Clinical Obstetrics and Gynecology*, for an issue on the antenatal and perinatal influences on the fetus and the newborn. The chapter demonstrates that the apparent influence of maternal height or birthweight is dissipated in the presence of the characteristics of maternal weight gain and prepregnancy weight. The paper also demonstrates the association of these latter characteristics of the mother with perinatal mortality.

   A revision of another manuscript, dealing with the influence of mother's smoking on the maternal weight gain, prepregnant weight association with birthweight, is near completion.

9. One-year neurological study. At the request of PRB, very extensive tabulations have been provided to indicate perinatal antecedents for one-year neurological outcomes. This study may result in another major document in the series of descriptive reports of the Collaborative Study data.

10. Eight-year speech, language and hearing examination. A preliminary study of the relationship between the 8-year examination and other test results has been designed and the data tabulated; the results are now being analyzed. This study will provide some insight into the sensitivity of items on the 8-year examination to variations in education of gravida, I. Q. at 7 years, and birthweights.

11. Neurological outcome at one-year and condition at 4 years. In collaboration with an investigator at Columbia University, a study has been made of repeat pregnancies of the same gravida, where a specified neurological condition is found in one child and no abnormalities are found in the other. Outcomes for the pairs of children at 4 years were compared, to identify important neurologic factors.

12. Consolidation of Collaborative Perinatal Study files. The records of the 60,000 women and their children of the Collaborative Perinatal Study preempt almost a full floor of space in the Federal Building. When the final records have been obtained and transferred to computer tapes, miniaturization of these records to conserve space and to reduce the number of file clerks required in their maintenance would be useful. The Office of Biometry is currently investigating many practical alternative methods for storing the raw data in a restricted space, at moderate cost, subject to rapid retrieval.

13. Speech, Language and Hearing Task Force. The Office of Biometry provided frequency tabulations of various outcomes and outcome combinations of test items in the three-year speech, language and hearing tests. Preparation of correlation matrices of speech, language and hearing variables and perinatal variables is underway. These computations are preliminary to the development of global scores for the three-year speech, language and hearing examination.

14. Techniques of non-orthogonal data analysis. The Office of Biometry is studying the suitability of non-orthogonal analysis of variance as a broad spectrum tool for analyzing Perinatal Collaborative Project data. Two broad approaches were considered: (1) the use of Markov's theorem on an appropriate model; (2) a synthesis of the main effects and interactions by linear combinations of corresponding contrasts within finely divided cells. The first approach appears more fruitful and is still under investigation with respect to: (1) conceptual problems, (2) computational problems, (3) its use by medical personnel.

15. Research on multiple regression. An improved statistical model in multiple regression is under study in collaboration with a statistical consultant. Tests, using data on a multiple regression predicting birthweight, did not show marked improvement. A new approach is under consideration.

16. Drug consumption in pregnancy and fetal malformations. This project has been funded under a contract with NINDS for the purpose of examining the data available from the Perinatal Research Branch on drug intake and fetal abnormalities. A member of OB is the assistant project officer under this contract, and acts as statistical consultant and advisor to the project.

17. Data processing support. To meet the extensive data processing requirements of the Perinatal Research program, the Section on Systems Design and Data Processing provides six full-time systems analysts and six full-time programmers to the Collaborative Perinatal Project. In addition to maintaining the massive data file systems of the Perinatal Research Branch these staff members supervised the Section on Data Management of the Branch, and provided the review, systems analysis, and data processing specifications for over 60 data requests, as well as administrative data for the management of contracts and various specialized programs. A list of some 30 of the more important of these requests follows:

    1. Mental Retardation in Relation to Post-Natal Events
    2. Monograph II
    3. Evaluation of 8 Year Speech, Language and Hearing Exam
    4. An Analysis of Epidemiological and Clinical Toxemia of Pregnancy - Perinatal Mortality
    5. Dilatation Study
    6. Recidivism in Pregnancy
    7. Demographic Tables - 4 Year and 7 Year Examination
    8. Physical Growth Study
    9. Intramural Quality Control Sampling Plan
    10. Additional Tables - Basic Document
    11. Inter Correlation of 4 Year Exam Scores
    12. Definitive Analysis of Prenatal Factors as they affect Neurological and Psychological outcomes in Children
    13. Case-finding, Negro mothers with sickling
    14. Gestational age distributions
    15. I. Q. Scores by Socio-Economic Intervals
    16. Protracted Descent - Labor and Delivery Study
    17. Regression Analysis - 4 and 7 Year Exam Scores
    18. Twin Analyses
    19. Infants with single umbilical artery
    20. Genetics of Obstetrical Variables
    21. Distribution of Children Dying by Type of Death
    22. Genetics of Obstetrical Variables
    23. Effects of Parity on Outcome of Pregnancy
    24. Labor Drugs and Anesthesia

25. Highest Total Serum Bilirubin of Newborn
26. Edema and Clinical Toxemia
27. Edema and Proteinuria
28. Toxemia of Pregnancy
29. Express III Program - 8 Year Special Language and Hearing Data
30. One Year Neurological Frequencies

EXTRAMURAL PROGRAMS

1. Urokinase-Pulmonary Embolism Trial. A report of Phase I results of the Urokinase-pulmonary embolism trial (NHLI) for establishing the thrombolytic effects of urokinase was reviewed. Recommendations were made to the Associate Director.

2. Statistical consultation and assistance were provided in the preparation of a report on manpower training requirements showing the effect of Training Grants and Award Programs of NINDS in the neurological field.

3. General data processing support for extramural programs.

   (a) The Office of Biometry assisted in the design and production of a form for a survey on training support of professionals in neurology. The office also carried out the data processing for tabular presentation of the results.

   (b) The Office of Biometry provided key punching specifications and advice on data processing to the Data Analysis and Reports Officer.

INTRAMURAL PROGRAMS

SURGICAL NEUROLOGY BRANCH

1. T-scope perception test. The Office of Biometry provided consultation on, and analysis of data from, a visual test module given to both right and left temporal lobectomy patients and a group of normal controls via a tachistoscope. Four tests were given to test short-term memory, perception, and learning. Chi-square analyses were used to determine differences between scores achieved by right and left temporal lobectomy patients and normals for these tests.

2. Experimental head injury. Rats were (1) sham concussed, trained, and tested 24 hours later, (2) trained, concussed, and retested 24 hours later, (3) trained, concussed, and immediately retested, and (4) concussed, trained and retested 24 hours later. The median test and distribution-free ANOVAS were used to determine (1) differences between training latency and retest latency for the four groups; (2) differences between the four groups for latency. This work is to be published in a paper, "The Effect of Experimental Head Injury on One-Trial Learning in Rats".

3. Growth hormone study. The Office of Biometry provided statistical services in this study of the effects of a growth hormone on children with organic (brain tumor, etc.) and ideopathic pituitary volumes. Ranges, means, standard deviations, and t-tests were used to determine whether the above groupings differentiated pathological volumes of the pituitary. This project was conducted in collaboration with the National Pituitary Agency. A paper, "The Collaborative Study of the Effects of Growth Hormone", is to be published.

4. Thalamotomy study. Numerous test variables were obtained on Parkinson patients treated with thalamotomy, preoperatively and post-operatively (from 1-10 year followups). The Office of Biometry set up the system for abstracting and tabulating the data for a punched-card file (including code lists, code sheets, sorting, counting, listing, etc.) The data has been and will be analyzed from four viewpoints - (1) clinical, (2) qualitative, (3) quantitative, and (4) intellectual and/or psychological. Paired t-tests, sign tests, ANOVAS, graphs, factor analyses, etc., have been and will be used to evaluate the patients preoperatively, immediately post-operatively, and at each year post-operatively. A series of at least 3 papers will be published on this study.

5. Effect of neurosurgery on the ability to identify popular word associations. The Office of Biometry consulted on the statistical analysis to be used in a study of the effect of temporal removals and subcortical operations on the ability to identify popular word associations.

6. Statistical problems in psychological tests.

   (a) Statistical assistance was given in revision and reprogramming of an empirical method of transforming test scores to normally distributed variables with fixed mean and standard deviation. The importance of using a sufficiently large sample of test scores to obtain a consistent and representative empirical distribution was emphasized.

   (b) The Office of Biometry consulted on a least-squares fitting of a bivariate polynomial equation for converting WAIS raw scores to age-corrected scaled scores in a research with Wechsler's adult intelligence scale.

7. Brain nuclei model. Various cross sections of monkey brains were used to create a model of the nuclei. These sections were distorted in normal laboratory processing.

   A master computer tape file was prepared from the output of the Calma Digitizer which read the original tracings. The IBM/360 was programmed to remove the distortions. The file created in this manner was processed to provide input to the CalComp Cathode Ray Plotter. The undistorted tracings obtained were used to make glossy prints for use as a camera copy in the publication.

MEDICAL NEUROLOGY BRANCH

1. Human muscle study. The Office of Biometry analyzed the data on the chemistry (pyruvate, succinate, beta hydroxy butyrate, palmitate, and creatine) of human muscles with respect to the red-white fiber ratio (count and weight), the patient's age, height, weight, and sex. The relationships between the chemistry and age, height, weight, sex, and creatine (also creatinine and percent creatine/creatinine), were examined by means of correlations, and regression analyses. To determine whether there were differences in the chemistry for discharge diagnoses (cerebellar involvement, anterior horn, normal and carriers, etc.), ANOVA's were carried out.

LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES

1. Glycogen levels in brain, liver and heart. Statistical consultation and assistance were given in a study of the mechanisms controlling glycogen levels in normal brain, injured brain, liver and heart of ischemia-, asphyxia- induced and control mice. Experimental data were analyzed by ANOVA, dose-response regressions, and testing significance of differences in mean response of individual or combined dosage levels. Analytical results were discussed and interpreted with the project scientists. Final draft of the manuscript for publication was reviewed for correctness of statistical presentation and interpretation.

2. Glycogen synthesis. Statistical consultation and assistance has been given in a neuropathological research project involving the development of assay procedures for glycogen synthesis. Tracer technique is to be employed in this assay. Numbers of photons released from C^{14} samples are to be counted to provide basic data in estimating sample disintegration rate. The statistical problem involves the derivation of calibration curves from the channel ratio method of quench correction. The experiment was done by counting variously quenched samples, containing known amounts of radioactivity, in two appropriate channels. The mathematical form of the standard curves relating counting efficiency to the ratio of net count rates in a sample in two different channels was constructed and fitted to the experimental data. A computer program for a non-linear least-square procedure was prepared for estimating parameters involved in the mathematical equations.

3. Animal weight and cell areas.

   (a) Data on cynomolgus and speciosa weights and areas of postramus mast cells have been collected. To determine the relationship between animal weight and cell areas, correlations, regression analyses, ANOVA's, and covariance analyses were performed and interpreted.

   (b) A second analysis, as above, was accomplished with the addition of more experimental data.

ELECTROENCEPHALOGRAPHY BRANCH

1. Psychiatric and personality associations in epilepsy patients.

   (a) Statistical analyses were completed for the paper, "Psychological and Neurological Comparisons of Psychomotor and Non-Psychomotor Epileptic Patients", which has been published in Epilepsia.

   (b) The Office of Biometry developed a data processing system for analyzing data collected to determine the predictive values of pre-operative EEG's, focal and non-focal ECoG's, etc. and for differences between unilateral and bilateral electrographic and clinical follow-ups for lobectomy, gyrectomy, and nontemporal patients. The data were summarized and analyzed by use of the information theory method of analyzing 3-factor contingency tables. A paper will be prepared on the work of this project.

2. Cerebral ischemia in monkeys. Data on impaired vascular filling after occlusion in monkeys was collected to show (1) whether occlusion is associated with impairment, treatment, and survival time, (2) whether survival time is associated with the character of vascular filling, and (3) whether clinical deficits and size of infarcts are different in treated and control groups. Chi-square analysis, ANOVA, and t-tests were carried out. This work is to be published in a paper "Impaired Microvascular Filling After Focal Cerebral Ischemia in Monkeys".

PERINATAL PHYSIOLOGY BRANCH

1. Primate research. Statistical analyses were carried out for a research paper entitled "Prediction of Gestational Age and Birthweight in Rhesus Monkeys (Macaca Mulatta)". Statistical methods involved stepwise multiple regression using higher order and interaction terms, correlation analyses based on Fisher's transformation, prediction "intervals" for regression surfaces, and nonparametric tests of the assumptions underlying regression techniques. Important results, some of which may have some bearing on the direction of primate research in the future are: (1) conception rates are higher when prepregnant uterus size is larger than normal, (2) gestational age can be predicted accurately in terms of either palpated uterus width or fundus height, (3) more than 50% of the variation in birthweight can be predicted by prepregnant weight, maternal weight gain, and gestational age at birth. The results should prove helpful in predicting gestational age of the Rhesus fetus and in controlling birthweight for Cesarean births required by the experimental design.

2. Behavioral study of the Rhesus monkey. Two groups of Rhesus monkeys were studied for behavioral relations among groups of free-ranging Rhesus monkeys; one group yielding records of about 27,000 grooming bouts between two Rhesus monkeys and the other involving some 3000 bouts. The Office of Biometry designed card layouts and coding forms, and punched cards and transferred them to magnetic tape yielding an

extensive data base. The data processing procedures have been structured for the application of more involved statistical procedures. To date, computer programs have been written to edit the data base and to provide early tabulation. Specifications for additional programs have been completed and the programs are currently being written. This project is expected to shed significant light on the social behavior of the Rhesus monkey and, as a consequence, determine potential areas for research in which behavioral studies of the Rhesus monkey might have direct bearing on human behavioral analyses.

3. Proposal to study the relationship between neurological symptoms and structural brain defects. Consultation was given on the design of a retrospective study, which involves a survey of neurological symptoms among mental hospital patients. The objective is to relate these symptoms to brain abnormalities discovered at autopsy, as patients eventually die. This complex, long range study would require a great deal of planning, and a very careful scrutiny of all data collected.

4. Effect of hypoxia on brain damage in rhesus monkeys. The investigation is concerned with describing some of the physiological and metabolic changes associated with a period of subtotal oxygen deprivation (hypoxia) in the rhesus monkey. The basic interest is in the response of the brain to hypoxia.

   The various measurements were coded for use in the computer, punched on IBM cards, verified, and subsequently punched onto paper tape for use on time sharing computer facilities.

   Data analysis is currently underway exploring the use of Jugular Oxygen Recovery Time (JORT) as a physiological measure of the experimental insult. The next goal will be a determination of the oxygen content in the blood as a function of some of the collected variables. The results will be used to determine future research.

LABORATORY OF MOLECULAR BIOLOGY

1. Protein Synthesis Rates. Mathematical consultation was provided in the comparison of protein synthesis rates of messenger and ribosomal RNA. With l_1 and k_1 denoting the rates of protein synthesis for messenger and ribosomal RNA respectively, M and N denoting their respective protein accumulations, and l_2 and k_2 their respective decomposition rates, the following equations hold:

$$\text{(i)} \quad M = l_1 \, (1-\exp(-l_2 t))$$

$$\text{(ii)} \quad N = k_1 \, (1-\exp(-k_2 t)),$$

   where the variable t symbolizes time. From data gathered experimentally, values were obtained for M/N, l_1/k_1, and l_2. An iterative computer method was developed to solve for k_2 in the equation resulting from the division of equation (i) by equation (ii). The estimate of l_1/k_1 was approximately 9/1 which differs significantly from the value 7/3, which is currently and possibly incorrectly assumed in the literature.

GENERAL PROCESSING SUPPORT FOR INTRAMURAL RESEARCH

1. The Office of Biometry provided file construction and maintenance and data processing for a study in monkey group behavior.

2. The Office of Biometry debugged and converted an IBM 7094 factor analysis program to the IBM 360 for the Section on Clinical Psychology.

GENERAL SUPPORT SYSTEMS FOR NINDS

1. The Office of Biometry developed and maintains a statistical and data processing reference file using the Key Word in Context Reprint Files (KWIC) system.

2. OB has developed a Datagraph System for the CalComp plotter which will permit individual researchers at NIH to obtain, at remote time-share consoles, graphs (point plottings, curves of best fit), and tabulations of statistical and mathematical parameters. The System has been simplified to the point where non-professional personnel, after a brief period of training, can operate the System. The System has been announced to NIH by means of DCRT publication, and several Institutes have requested the service. An NINDS seminar on the System is in the planning stage.

3. A graphic manual has been developed for potential users and will be distributed.

4. OB has set up a system that enables it to access any set of data in the DCRT computer system and convert it to paper tape output to be used as input to the time-sharing system. This system is of particular value to OB programmers and statisticians.

5. Modified IRS (the DCRT computer program) in the NIH tape library to facilitate the production of lists and tables.

6. The data checking program will be modified, under contract. It provides for computer file creation, and in the process edits data for accuracy.

7. The Express III program package was developed further for greater flexibility, and to provide for the production of more complex tables.

8. IBM is preparing a report on its study, under contract, of the entire Collaborative Project file structure, with special emphasis on the applicability of new techniques for file storage.

9. OB has increased the capacity for the use of computer remote stations by programmers, program analysts, and statisticians. These 20 remote stations in OB have become critically important instruments for programming and for statistical analysis. The costs of these remote station rentals are fully covered by the payments, by other Institutes, for the use of NINDS computer equipment.

10. A member of the Office of Biometry serves on the Contract Review Committee of the Cancer Institute, and provides consultation to non-governmental medical research organizations.

11. Computer programs for time sharing systems were prepared as follows:

    a. A Basic program ranks numbers, prints and/or stores numbers and ranks in original and/or rank order.

    b. A Basic program computes rank correlation matrix and product moment correlation matrix for P groups of N numbers.

    c. A Basic program and a PL1 program were prepared for use in sequence. These programs will perform automatic coding of titles and legends for the SBC Call/360 plotting routines.

    d. A Basic program groups data in x-axis intervals of chosen width and provides frequency values for a frequency plot using the SBC Call/360 plotting routine.

    e. Two programs in Basic language for the manipulation of large binary data files. This involved either automatic editing and updating of file data or condensing the basic number of computer spaces required for data storage.

OTHER ORGANIZATIONS

NATIONAL INSTITUTE OF CHILD HEALTH

1. Auditory stimulation and cortex cell response. Statistical consultation was provided to a NICHD scientist at the recommendation of a former NINDS neurophysiologist in a study of cortex cell response to auditory stimulations with squirrel monkeys. The experimental work involved recording 13 different monkey calls of known frequencies such as peep, tweet, arr, etc., on a tape recorder; these calls were then played back to experimental monkeys with microelectrodes implanted in various cells of their cortex. Response or nonresponse to each call was observed. Statistical work involved analyzing the auditory responses from a large number of tests to investigate the existence of clusters in these calls. A clustering procedure based on binomial probability distribution was developed for this purpose. A computer program was prepared to carry out the statistical computations. Results of the analysis was discussed with the investigator and recommendations were made on further work.

INSTITUTE OF PUBLIC HEALTH, BELGRADE, YUGOSLAVIA

1. Maternal nutritional supplement study. The final proposal for a maternal nutritional supplement study in a deprived population in Serbia has been developed jointly by the Institute for Health Protection, Belgrade, Yugoslavia, the Perinatal Research Branch, and the Office of Biometry. The research proposal is aimed at testing the effects of nutritional deprivation on the perinatal mortality rates and neurologic sequellae of the infants.

   A site visit was made to a number of the villages from which the study population would be selected, and a wide variety of nutritional supplements were tested for taste acceptability.

   The research proposal is ready for ad hoc committee review. The Chief of the Office of Biometry has been invited to be a Co-Project Officer.

NATIONAL INSTITUTE OF MENTAL HEALTH

1. L-dopa and WAIS tests. The Office of Biometry provided consultation on the Wilcoxon matched-pairs signed-ranks tests and on Chauvenet's Criterion (chance extremes, expressed as Studentized deviates, can be eliminated by excluding, in relation to the number of measurements, all values greater than $(1/\sigma)(X \underset{\sigma}{-} \bar{X})$). It was suggested that the use of the Wilcoxon test, as already applied to the L-dopa and WAIS tests data, was appropriate.

NATIONAL EYE INSTITUTE

1. Bachet's syndrome. The Office of Biometry provided consultation on the methods of analyzing differences between data from tissue from patients with Bachet's syndrome and normals. The recommendation was that these data should be analyzed by ANOVA's, t-tests, etc., preferably by the Office of Biometry, N.E.I.

DIVISION OF RESEARCH GRANTS & FOOD & DRUG ADMINISTRATION

1. Instruction was given in the use and the programming of the Mathatron and the Wang 700.

REVIEW COMMITTEES

The Office of Biometry has been represented on a number of ad hoc review committees:

1. NIAID - The Chief, OB, chaired a committee which reviewed a contract proposal for the development of diagnostic criteria for certain allergies.

2. NIGMS - The Chief, OB, was co-chairman of an ad hoc committee in the review of a contract for the clinical pharmacology department of Tufts University.

3. DRG - The Office of Biometry participated in the review of a contract to measure effect of duration of postpartum hospitalization on child health.

RESEARCH

1. The researcher is sometimes faced with the problem of deciding whether or not the elements of a row or column of a two-way layout are sampled from a population with the same variance as the rest of the rows or columns. To date, the results in the literature dealing with this problem give tests which are based on large sample approximation theory.

   An exact test is being developed, which can be used for small samples, and a paper is to be submitted for publication in the next year.

2. Research is underway to estimate the power of a test for outliers. The test can be used for multivariate regression problems, and the power of the test will be based not only on the sample size and size of the outlier, but also on the correlation structure of the error estimates from the linear model. These results should present many insights into the theory of linear regression. A paper is to be submitted for publication within the next year.

3. A mathematical recurrence is derived for the exact multiple runs distribution and tables of critical values provided. The research was partly stimulated by an Office of Biometry consultation project which needed a randomness test for determining whether three data samples could be pooled. A paper on this subject will be submitted shortly for publication.

4. A statistical procedure was developed for selecting quantal response models. The mathematical derivation of this procedure has been completed. An empirical study of this procedure is to be carried out for demonstrating its efficiency of selecting the correct mathematical model. Report of the research results will be prepared for publication.

5. In multiple comparisons, the sample mean from the population with the largest sample mean is not an unbiased estimate of its population mean. Analytical expressions have been derived for estimates of means of normal populations when these are chosen on the basis of the ranks of their respective sample means. A Monte Carlo program has been developed to verify and supplement the theoretical analysis. Some of the estimates investigated have (1) suitably small bias, (2) relatively large variances; (3) smaller mean square errors than the conventional estimates that ignore the choice of the population

on the basis of sample ranks. Current work is directed to estimates with smaller variances. The results of this research may have wide applicability throughout NIH. A talk on the broader subject of the estimation of parameters of selected populations was given in an NIH statistics seminar.

MAJOR PLANS FOR FY 1972

1. Continue to provide statistical and mathematical service to the research branches in the Intramural and the Collaborative & Field Research programs, and to other Divisions of the Institute.

2. Increase research activities in the following areas:

   (a) A systematic investigation and research into the methodology of analysis of neuronal spike train data.

   In many neurological investigations, the train of impulses ("spikes") produced by a nerve cell (or cells) is subjected to a statistical treatment involving the time intervals between spikes. This statistical treatment falls into the area of stochastic point processes. Due to the importance of this type of experimentation in the Institute, the Office of Biometry should develop a proficiency in the area of stochastic point processes in order to be of greater service to the Institute scientists. A comprehensive survey of the literature has been undertaken. Preliminary work has been undertaken to simulate various spike trains in order to gain insight into the meaning of various statistical measures of the train. Data from some laboratories of the Institute are available and will be used in subsequent analyses in cooperation with Institute scientists. In addition, an International Conference on Stochastic Point Processes is to be held this summer in Yorktown Heights, New York. The purpose of the Conference is to inform scientists of the most recent ideas and developments concerning the application of stochastic point processes to various real world problems, including neurophysiological experimentation. All of these opportunities will speed the learning process involved and enable the Section on Mathematical Statistics to proceed more quickly to the analysis of the data generated in this scientific area.

   (b) Examination of the time sequence of epileptic seizures.

   A survey of the literature on epilepsy (Epilepsy Abstracts) has shown a paucity of hard data on the clustering of epileptic seizures. The question still remains unanswered as to whether a burst of seizures in one time period leaves an epileptic more or less vulnerable to additional seizure activity in the following period.

The Directors of the New Castle State Hospital for epileptics have made available their entire patient file. These files have been extremely well maintained over a number of years, and contain valuable medication and seizure activity information.

The Section on Mathematical Statistics plans to derive a methodology for a data file, which will allow for the elimination of extraneous and biasing factors. A mathematical model will be derived describing stochastic mechanisms involved in the timing of the type of seizures experienced at New Castle.

(c) Investigation and research into the statistical methodology of analysis of serological experiments.

The specific nature of the experimental measurements in serological research should require new or refined statistical techniques in data analysis in addition to the conventional method now available. However, there does not seem to appear in the statistical literature a comprehensive evaluation of the appropriateness of various statistical methods now adapted to serological research. A study plan is under consideration which will investigate the present status of the application of statistical methods in this area. Further research will be done in the development of new or improved statistical techniques wherever necessary. A comprehensive survey of the literature will be undertaken by this Section. Research effort will first be concentrated on a recording system of serological titers and distributional properties of this type of measurements.

3. Develop a proposal for research into the long-term safety and efficacy of L-dopa in Parkinson's disease. If the study is developed, Office of Biometry would be interested in participating in this research.

4. The Office of Biometry will participate in the preparation of a monograph relating prenatal factors to the neurological and psychological outcome of children from the Collaborative Project, by the Four and Seven-Year Neurological and Psychological Task Force.

5. In collaboration with the Department of Neurology, UCLA, a book will be written on Clinical Quantitative Neurological Testing.

6. The Office of Biometry seeks to promote fuller utilization of computers by further developing user-orientated software products for non-programmers.

MISCELLANEOUS ACTIVITIES

1. Seminar on clinical quantitative neurological testing. A seminar on clinical quantitative neurological testing was presented at NINDS by members of the Department of Neurology, University of Michigan, and a member of the Office of Biometry.

2. Seminar on estimation of parameters of selected populations. A member of the Office of Biometry presented a paper at the NIH Statistics Seminar on the estimation of parameters of selected populations.

3. Referee Services. Members of the Office of Biometry reviewed papers for *Nature* and *Technometrics*.

4. Review of papers. The Office of Biometry reviewed numerous papers written by NINDS personnel as well as by persons from other organizations.

PUBLICATIONS

1. Horta-Barbosa, L., Krebs, H., Ley, A., Chen, T.C., Gilkeson, M.R., and Sever, J.L.: Progressive increase in cerebrospinal fluid. Measle antibody levels in SSPE. J. Pediat. 78: 1971.

2. Friedman, E.A., and Kroll, B.H.: Computer analysis of labor progression. II. Distribution of data and limits of normal. J. Reprod. Med. 6: 43-48, 1971.

3. Friedman, E.A., and Kroll, B.H.: A use of the computer in the analysis of labor progression. Presented at the 9th Annual Symposium on Biomathematics and Computer Science in the Life Sciences, 1971.

4. Mersereau, L.R., Kroll, B.H., and Smith, D.C.: A large scale graphics system. Technical note in Proceedings of the American Federation of Information Processing Societies, Spring Joint Computer Conference, 1971.

5. Mignone, R.J., Donnelly, E.F., and Sadowsky, D.: Psychological and neurological comparisons of psychomotor and non-psychomotor epileptic patients. Epilepsia. 11: 345-349, 1970.

6. Sever, J.L., Gilkeson, M.R., Chen, T.C., Ley, A.C., and Edmonds, D.: Epidemiology of mongolism in the collaborative project. Ann. N.Y. Acad. Sci. 171: 328-339, 1970.

7. VanBuren, J.M., Henderson, W.G., Sadowsky, D., Shapiro, D.Y.: A qualitative and quantitative evaluation of Parkinsonians three to six years following thalamotomy. Presented at the meetings of the American Association of Neurological Surgeons, April 18-21, 1971 in Houston, Texas.

8. Friedman, E.A. and Kroll, B.H.: Computer analysis of labor progression. II. Pattern variations by parity. J. Reprod. Med. 6: 63-67, 1971.

Table of Organization

Intramural Research

National Institute of Neurological Diseases and Stroke
(Personnel on hand May 1971)

Office of the Director

Director of Intramural Research - Henry G. Wagner, M.D.
Assistant Director of Intramural Research - Richard L. Irwin, Ph.D.
Act. Clinical Director - John M. Van Buren, M.D., M.Sc.
Administrative Officer - Glenn E. Hammond
Mathematician - Rosalind B. Marimont
Senior Staff Fellow - William K. Stell, M.D., Ph.D.
Research Psychologist - Margaret Varley, Ph.D.
Research Biologist - Halsey M. Marsden, Ph.D.
Administrative Officer - J. Loring Jenkins
Photographer (Scient. & Tech.) - Wesley Pearson, Jr.
Engineering Technician - Oscar M. Wiseman
Photographer (Medical) - Jerry M. Ellis
SA Surgeon - Michael O. Salcman, M.D.
Biological Laboratory Technician (Animal) - George R. Duvall
Biological Laboratory Technician (Animal) - Adrian P. Loftis
Photographer (Medical) - Norman E. Mills
Secretary (Steno) - Margret L. Shipley
Special Assistant - Nancy F. Nusbaum
Travel Assistant - Ida M. Chernikoff
Procurement Clerk - Doris R. Perry
Clerk (Dict.Mach.Trans.) - Kathleen Knapowski
Histopathology Technician - Thelma R. Fletcher
Budget Clerk (Typing) - Heather A. Roberts
Clerk-Stenographer - Lynne H. Minnick
Laboratory Worker - John R. Bowers
Laboratory Worker - Herbert D. Inabinet
Laboratory Worker - Kenneth M. Oglesbee

Section on Technical Development

Computer Programmer - William H. Sheriff, Jr.
A San E - George M. Carman
Engineering Technician - Benjamin H. Shellman

Medical Neurology Branch

Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Assistant Chief - John R. Warmolts, M.D.
Biological Laboratory Technician (Microbiol.) - Guy G. Cunningham
Biologist - Donald W. Bishop

Physiologist - E. Carolyn Derrer
Surgeon - Dennis L. Martin, M.D.
Surgeon - John N. Whitaker, M.D.
Surgeon - Jerry R. Mendell, M.D.
Surgeon - Alan Borenstein, M.D.
Surgeon - Alan M. Rapoport, M.D.
Surgeon - Robert I. Roelofs, M.D.
Surgeon - Robert J. Schwartzmann, M.D.
Surgeon - Peter Tsairis, M.D.
Secretary (Steno) - Vernita Bergmeyer
Biologist - Mary A. Oberc
Clerk (Dict.Mach.Trans.) - Lucy L. Riley
Secretary (Dict.Mach.Trans.) - Gertrude E. Wright
Biological Laboratory Technician - Pauline R. Middleton
Microbiologist - Priscilla B. Chauvin
Clerk (Dict.Mach.Trans.) - Anne K. Bowen
Biological Laboratory Technician - Daniel L. Baker
Laboratory Worker - Matthew P. Meadows
Clerk-Typist - Beverly Williams

Section on Clinical Applied Pharmacology

Super. Research Pharm.(Gen) - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Biologist - Katharine L. Oliver
Secretary (Dict.Mach.Trans.) - Emma P. Dick

Surgical Neurology Branch

Office of the Chief

Act. Chief, Surgical Neurology Branch - John M. Van Buren, M.D., M.Sc.
Medical Officer (Neuro Surg) - Choh-luh Li, M.D.
Medical Officer (Neuro. Surg) - Ayub Khan Ommaya, F.R.C.S.
Research Psychologist (Clin) - Paul Fedio Jr., Ph.D.
Research Physiologist - L. Claire Parsons, Ph.D
Medical Officer - Charles R. West, M.D.
Visiting Scientist - Susumu Nakatani, M.D.
Biologist - Rosemary C. Borke
Supervisory Operating Room Nurse - Frances D.S. Lamberti. R.N.
Supervisory Operating Room Nurse - Mildred L. Haddox, R.N.
Operating Room Nurse - Delta H. Trickett, R.N.
Operating Room Nurse - E. Dolores Blessing, R.N.
Biologist - Levon O. Parker
SA Surgeon - Frederick A. Matsen, M.D.
Surgeon - Kalmon D. Post, M.D.
Surgeon - John P. Franz, M.D.
Surgeon - Thomas A. Gennarelli, M.D.
Surgeon - Sherman C. Stein, M.D.
Biological Laboratory Technician (Animal) - Mitchell A. Major
Biological Laboratory Technician (Animal) - M. Arthur Banks

Psychologist - Mary H. Spear
Secretary (Steno) - Flora P. Feld
Clerk (Dict.Mach.Trans.) - Helen M. Andregg
Clerk-Stenographer - Dawn M. Weber
Secretary (Steno) - Michele Y. Shevitz
Health Technician - Morris M. Williams
Health Technician - Howard F. Stewart, Jr.
Psychology Technician - Christiane S. Cox
Zoologist - Charles G. Morrow
Biological Aid (Microbiology) - Joceline L. Ash
Medical Aid - Octavie W. Jacobs
Clerk-Stenographer - Constance C. Hampton

Section on Primate Neurology

Biologist - Kenneth Rich
Health Technician - Charles E. Sartor
Biological Laboratory Technician (Animal) - Leo Jacobs
Biological Aid (Animal) - Clifford A. Seay

Section on Neuroanesthesiology

Biological Laboratory Technician (Animal) - Calvin S. Hawkins

Section on Clinical Psychology

Super. Research Psychologist - Herbert C. Lansdell, Ph.D.
Secretary (Typing) - Lillian L. Wease

Section on Child Neurology

Medical Officer (Neuro) - Anatole Dekaban, M.D.
Research Chemist - Dennis F. Cain, Ph.D.
Research Chemist - George Constantopoulos, Ph.D.
Medical Technician (Chem.) - Jan K. Steusing
Surgeon - Kenton R. Holden, M.D.
Secretary (Steno) - Sandra S. Costanzo
Biological Aid (Biochem.) - Milton H. Louie
Biological Aid - Edward D. Ball

Section on Neuroradiology

Research Medical Officer - Giovanni Di Chiro, M.D.
Clerk-Stenographer - Dianne L. Beall

Electroencephalography Branch

Office of the Chief

Chief, EEG Branch - Cosimo Ajmone Marsan, M.D.
Surgeon - Michael J. O'Conner, M.D.
EEG Technician - Rosemarie Schilling

EEG Technician - Alice G. Spencer
Secretary (Dict.Mach.Trans.) - Mattie L. Jackson
Clerk-Typist - Carol L. Tabb

Section on Clinical Neurophysiology

Surgeon - Jeffrey L. Barker, M.D.
Surgeon - Marc A. Dichter, M.D., Ph.D.
SA Surgeon - Chester J. Herman, M.D., Ph.D.
Biologist - John H. Jones

Laboratory of Neuropathology & Neuroanatomical Sciences

Office of the Chief

Chief, LNNS - Igor Klatzo, M.D., M.Sc.
Surgeon - Andrew A. Zalewski, M.D.
Biological Laboratory Technician (Animal) - Willie Perkins
Biological Laboratory Technician (Animal) - Albert V. Cantu
Biological Laboratory Technician (Animal) - Melvin H. Carroll
Secretary (Dict.Mach.Trans.) - Virginia A. Masterson
Clerk (Typing) - Hilda M. Malcolm
Clerk (Typing) - Mary D. Wallace
Visiting Fellow - Hiroshi Watanabe, M.D.

Section on Trophic Nerve Function

Medical Director - Lloyd Guth, M.D.
Physiologist - Herbert Yellin, Ph.D.
Biological Laboratory Technician (Microbiol.) - Janina D. Ziemnowicz
Biologist - William C. Brown
SR HSO - George F. Creswell

Section on Neurocytology

Research Physiologist - Milton W. Brightman, Ph.D.
SA Surg - John E. Heuser, M.D.
Biological Laboratory Technician - Clara E. Mowry
Biologist - Marie A. Shatos
Visiting Fellow - Sachio Nabeshima, M.D.

Section on Functional Neuroanatomy

Medical Officer - Thomas S. Reese, M.D.
Biological Laboratory Technician - Frank D. Nolan

Section on Comparative Neuropathology

Biological Laboratory Technician - Joseph T. Walker, Jr.
Biological Laboratory Technician (Biochem.) - Ernestine G. Dye
Surgeon - David A. Rottenberg , M.D.
Visiting Fellow - Masaharu Hori, M.D.

Section on Experimental Neuropathology

Medical Officer (Path) - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Biological Laboratory Technician - Margaret G. Johnson
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

Section on Cellular Neuropathology

Medical Officer - Henry deF. Webster, M.D.
Biological Laboratory Technician - Maureen F. O'Connell
Biologist - John R. Martin

Section on Cellular Neurochemistry

Research Biologist - Janet Passonneau, Ph.D.
Staff Fellow - Wesley D. Lust, Ph.D.
Biologist - Helen J. Osborne

Section on Neurocytobiology

Medical Officer - Maria Spatz, M.D.
Biological Laboratory Technician (Micro.) - Madora E. Swink
Biological Laboratory Technician (Micro.) - Jo Ann Holmes

Laboratory of Biophysics

Office of the Chief

Super. Research Physiologist - Robert E. Taylor, Ph.D.
Senior Research Biophysicist - Kenneth S. Cole, Ph.D.
Research Physicist - Richard Fitzhugh, Ph.D.
Research Physiologist (Neurophys.) - Daniel L. Gilbert, Ph.D.
Electrical Engineer (Instru.) - Leonard Binstock
Research Physicist - Gerald M. Ehrenstein, Ph.D.
Mathematician - John Shaw
Research Physicist - Harold Lecar, Ph.D.
Electronic Technician - Herbert A. Walters
SR Surg - Irving M. Stillman, M.D. Ph.D.
Biologist - Allen D. Spencer, Jr.
Visiting Fellow - Ramon Latorre, Ph.D.
Clerk-Stenographer - Sandra J. Cotter
Biological Aid - Mark A. Holbert

Laboratory of Neurochemistry

Office of the Chief

Chief, Laboratory of Neurochemistry - Donald B. Tower, M.D., Ph.D.
Secretary (Steno) - Shirley A. Burdette

Clerk-Stenographer - Carol A. Bromley
Clerk-Stenographer - Michele A. Cahill

Section on Amino Acid Chemistry

Chemist - Oscar M. Young

Section on Physiology and Metabolism

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter

Section on Enzyme Chemistry

Research Chemist - R. Wayne Albers, Ph.D.
Chemist - George J. Koval
SA Surgeon - Robert C. Collins, M.D.
Chemist - Sally A. Sveda
Visiting Fellow - Dou H. Jean, Ph.D.
Physical Science Technician (Chem.) - Eunice L. Summers

Section on Lipid Chemistry

Head, Section on Lipid Chemistry - Roscoe O. Brady, M.D.
Research Chemist - Andrew E. Gal, Ph.D.
Chemist - Roy M. Bradley
Senior Staff Fellow - Richard H. Quarles, Ph.D.
Visiting Scientist - Rolf Sammeck, M.D., Ph.D.
Biological Laboratory Technician - Frank J. Fash
Chemist - Jane M. Quirk
Surgeon - William G. Johnson, M.D.
Staff Fellow - Peter H. Fishman, Ph.D.
Biological Laboratory Technician - Alexander S. Wheaton
Physiologist - Michael S. Glasgow

Laboratory of Molecular Biology

Office of the Chief

Chief, Laboratory of Molecular Biology - Ernst Freese, Ph.D.
Secretary (Steno) - Mary W. Taylor
Laboratory Worker - Theodore J. Grey
Clerk-Typist - Eileen Baker

Section on Developmental Biology

Research Biologist - Elisabeth G.M. Freese, Ph.D.
SR Scient - Norman L. Richards, Ph.D.
Surgeon - Eugene L. Speck, Ph.D., M.D.
Staff Fellow - Michael L. Hampton, Ph.D.
Staff Fellow - Martin D. Diesterhaft, Ph.D.

Chemist - Enid M. Gallers
Biological Laboratory Technician (Micro.) - Blanche E. Lewis
Visiting Fellow - Wilhelmus N. Konings, M.D., Ph.D.
Visiting Fellow - Yong K. Oh, Ph.D.
Visiting Fellow - Chingju Wang Sheu, Ph.D.
Visiting Fellow - Chandan Prasad, Ph.D.

Section on Regulation of Nucleic Acid Syntheses

Research Chemist - Robert A. Lazzarini, Ph.D.
Staff Fellow - Leonard E. Maroun, Ph.D.
Visiting Fellow - Yoshikatsu Murooka, Ph.D.

Section on Regulatory Cell Physiology

Medical Officer - Charles M. Cashel, M.D.
Chemist - Barbara Crowe

Laboratory of Experimental Neurology

Chief, Laboratory of Experimental Neurology - William F. Caveness, M.D.
Visiting Scientist - Motohiro Kato, M.D.
Biological Laboratory Technician (Animal) - Kenneth H. Hess
Secretary (Steno) - Dee Ann Weller
Clerk-Typist - Teresa M. McMullen
Clerk-Typist - Marguerite H. Smiley

Laboratory of Neurophysiology

Chief, Laboratory of Neurophysiology - Michelangelo G.F. Fuortes, M.D.
Electronic Technician - Vincent T. Almasy
Biological Laboratory Technician (Animal) - William L. Beane
Secretary (Dict.Mach.Trans.) - June M. Picone
Clerk-Stenographer - Anita T. Page

Section on General Physiology

Senior Research Neurophysiologist - Ludwig von Sallmann, M.D.
Chemist - Patricia A. Grimes
Surgeon - John S. McReynolds, M.D.
SA Surgeon - Eric A. Schwartz, M.D.
Visiting Fellow - Roberto Fioravanti, Ph.D.

Section on Sensory Physiology

Medical Officer - Thomas G. Smith, Jr., M.D.
Senior Staff Fellow - George C. Murray, Ph.D.

Section on Cell Biology

Research Biologist - Arnaldo Lasansky, M.D.
Biological Laboratory Technician (Micro.) - Julia M. Lohr

Section on Neuronal Interactions

Head, Section on Neuronal Interactions - Edward F. MacNichol, Ph.D.
Staff Fellow - Michael L. Wiederhold, Ph.D.
Electronic Technician - Ferenc Harosi

Laboratory of Neural Control

Office of the Chief

Chief, Laboratory of Neural Control - Karl Frank, Ph.D.
Medical Officer - Robert E. Burke, M.D.
Research Physiologist - Donald R. Humphrey, Ph.D.
Surgeon - Frederick T. Hambrecht, M.D., Ph.D.
Surgeon - David N. Levine, M.D.
Surgeon - Richard G. Jost, M.D.
Secretary (Steno) - Nell W. Winnie
Psychologist - Kathleen K. Davis
Electronics Technician - Martin J. Bak
Clerk (Dict.Mach.Trans.) - Cathleen A. Clarke
Visiting Fellow - Mahmood J. Nahvi, Ph.D.

Laboratory of Perinatal Physiology

Office of the Chief

Chief, Laboratory of Perinatal Physiology - Ronald E. Myers, M.D., Ph.D.
Research Chemist - Americo Rivera, Jr., Ph.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Engineering Technician - George M. Dold
Chemist - Jose Martinez de Jessus
Biological Laboratory Technician (Animal) - Esteban Monell-Torrens
Biological Laboratory Technician (Animal) - William Rodriguez
Surgeon - Myron D. Ginsberg, M.D.
Surgeon - Douglas Y. Shapiro, M.D.
Surgeon - Michael E. Selzer, M.D.
Surgeon - Michael J. Labance, M.D.
SA Surgeon - Dennis Selkoe, M.D.
Chemist (Biochemistry) - Carmen L. Freixas
Biological Laboratory Technician (Micro) - Maria L. Yamaguchi
Biological Laboratory Technician (Micro) - Luz Delia G. Salazar
Anthropologist - Elizabeth A. Franzen
Secretary (Steno) - Elsie M. Walter
Biological Laboratory Technician (Animal) - Joseph C. Pazdersky
Animal Caretaker - Bernardino Santana
Zoologist - Lawrence E. Dean
Clerk-Stenographer - Patricia A. Scafone

Annual Report of the Scientific Director of the National Institute of Neurological Diseases and Stroke

July 1, 1970 through June 30, 1971

The Intramural Research Program continues to be a productive and vigorous research effort directed at all aspects of the neurological sciences, including the clinical disorders. Forty-one new projects were started during the past year and 12 ongoing projects were completed. From a total of 158 active projects within the program, 56 are conducted with cooperating units located in universities or other research institutions outside the NIH. Forty-three projects are carried on with the collaboration of other research units within NIH. This intensive interaction of the Intramural Research laboratories with other research institutions indicates the degree to which our program has flourished during the past year in relation to the broader scientific community.

Some organizational changes important for thrusts in new directions took place within our laboratories during the past year, and others are planned. Sections on Cellular Neurochemistry and Neurocytology were activated in the Laboratory of Neuropathology and Neuroanatomical Sciences by the hiring of the senior investigators. Retirement of a key senior scientist adversely affected research in the Section on Functional Neuroanatomy. A Chief has been recruited to direct our biophysical studies and that laboratory will be reorganized. Other changes of program impact are being considered that will increase our capabilities to more effectively apply to clinical disorders the basic knowledge we have gained from neurochemistry studies. A new Section on Neural Interactions has been established in the Laboratory of Neurophysiology.

An important effort in neural oncology and another in neuro-otolaryngology have been proposed to start during the coming year. Neither can begin without additional funds, facilities and personnel positions. Other program initiatives that are unique to our capabilities and appropriate for the mission have been identified. These are, an effort to determine the possibilities of gene therapy and an investigation of the metabolic defects in tumor cells using the same experimental approaches that successfully uncovered the metabolic faults in Tay-Sachs, Gaucher's and a number of other familial diseases. These initiatives have been designated for future attention because of a high probability for making needed and significant contributions to the nation's health in the near future.

The pursuit for progress in the understanding and treatment of epilepsy has continued to be an important effort of our program and encompasses both clinical studies and laboratory experiments. Epilepsy with a frontal-centro-parietal localization has been studied to determine the localizing significance of the various sensory, motor and autonomic aspects of the seizure patterns. Results should prove of practical usefulness for topographical identification of the epileptogenic process and, indirectly, for its surgical removal. A further study of patients after temporal lobectomy shows that recovery of reaction time does take place and indicates some neural reorganization in the adult human central nervous system. Experimental efforts have helped define both the role of various neurons in the production of paroxysmal or rhythmical sustained activity and the amount of synaptic activity sufficient for the onset of typical epileptiform manifestations. Ketogenic diets have been found to induce synaptic and nerve changes in experimental animals that may relate to increased threshold of excitability and the studies may thus explain the usefulness of the high fat diets in the treatment of epilepsy. Other animal studies on the course of development of epileptiform seizures found that propagation of the paroxysmal discharge of the immature motor cortex depends on thalamic structure.

We have continued to apply basic research techniques in a multidimensional attack on certain neurological disorders. This supports our thrust toward a more precise definition of each abnormality and separation of each disorder into more distinct sub-forms which leads, hopefully, to the development of specific or symptomatic therapy. We have been able to formulate a new hypothesis on the pathogenesis of myasthenia gravis and have introduced a new and effective treatment for this disease by administration on alternate days of a high single dose of prednisone. A vascular abnormality has been found in skeletal muscle in the progressive pseudohypertrophic dystrophy of Duchenne that explains the pathogenesis of the disease. The development and use of an experimental animal model supported the hypothesis that the defect of this type of dystrophy is a vascular one. Other progress includes finding that the attacks of hypokalemic periodic paralysis are effectively prevented by the administration of acetazolamide. The development of a new open-biopsy technique for obtaining absolute electromyographic-histochemical correlations of normal and abnormal human muscle and the successful growing in tissue culture of human muscle fibers obtained by biopsy are encouraging accomplishments for future research.

The study of neurochemistry continues to provide a major thrust toward understanding the organizational aspects of the nervous system; particularly at the level of the neural membranes. Comparative, ontogenetic and maturation studies are designed to identify principles of structure and function that are often obvious only from careful developmental and interspecies comparisons. The unexpectedly high turnover rates of the basic portion of myelin makes untenable the long-standing concept of myelin as a stable structural element. These studies give promise of more successful attacks on the problem of demyelination in multiple sclerosis and related disorders. Myelination patterns have been studied in peripheral nerves since abnormalities in the pattern may be important factors in hereditary and developmental neuropathies. The data collected in this study is expected to be useful for interpretation of some abnormalities seen by use of the electron microscope and reported in human disease. More than a decade of complex research in lipid chemistry has brought to fruition some compelling examples of the application of basic research to clinical problems. Suitable diagnostic procedures have been developed for detection of sphingolipid storage diseases. Screening for heterozygous carriers and the monitoring of pregnancies at risk are now accomplishments of past research. Aminocenteses have successfully predicted normal fetuses and also those afflicted with Fabry's, Niemann-Pick, or Gaucher's disease. The knowledge gained from the glycolipid studies is believed to be generally applicable and has led us to embark on somewhat parallel investigations on glycoproteins, substances which subserve vital receptors and transport functions of cell surfaces.

Experimental approaches to brain tumors and allied problems have been developed in several program areas. Investigations on the chemotherapy of glioblastoma continues with emphasis on developing baseline indicators of a therapeutic response. The use of antitumor materials tagged with radioactivity has provided a more rational approach to chemotherapy and also furnishes the means for an objective index to response of the tumor. The achievement of a functional tomoscanner to be installed in the Clinical Center adds a new dimension in scanning precision that will greatly improve our capability in tumor detection. Another newly-developed instrument permits intratumor instillation of lipid soluble chemotherapeutic agents by percutaneous puncture. The device aids in the search for the specific tumor antigens which make possible the study of immunotherapeutic methods of prevention and treatment of glial tumors. Studies of normal electrical characteristics of both neurons and nonexcitable glia cells have been extended to tumor material. This permits evaluation of the differential effects of chemotherapeutic agents on normal and neoplastic components of the brain. Some fundamental studies have shown that in certain tumorogenic cells the biosynthesis of gangliosides is blocked. The defective enzymic step has been identified. Such tumorogenic cells have lost their normal cellular ability to inhibit the growth of other cells by contact, a property that may correlate with the presence of gangliosides. Spontaneous reversal to normality destroys the tumorogenicity of the changed cells and restores their contact inhibition properties. These studies are highly relevant to our knowledge of tumor mechanisms because they relate both to inhibition of cell growth and functional organization of cell membranes.

Spinal cord and cerebral vascular disease has continued to receive our attention. Experimental angiography has demonstrated complex patterns of ascending and descending blood flows. These new findings may be applicable to human diseases of the spinal cord. The continuing study of human spinal cord angiography has led to new criteria for obstructive vascular disease of the cord. Studies were undertaken to evaluate arterial crossover in patients with normal cerebral vasculature. These will provide data on collateral flow channels of possible importance in the susceptibility or resistance to stroke.

A number of investigations have emphasized enzymic mechanisms. An extensive study on the sodium and potassium activated ATPases provided important kinetic data on catalytic sites of the enzymes. Structural studies are defining the amino acid composition of the phosphorylated peptide of this transport enzyme. A comprehensive survey of the enzymes characteristic of plasma membranes has been completed. Other studies are directed toward the feasibility of trials of enzyme replacement therapy. Such therapy could remove a harmfully accumulating biochemical substance by furnishing to the patient a missing enzyme. A new system was developed for analyzing small samples of biopsied human muscle by use of radioisotopic biochemical assays. This has permitted a more precise differential examination of enzyme activity among muscle types and comparisons between normal and abnormal muscles.

The study of gestational problems affecting brain maturation has continued with one emphasis on abruptio placentae, a serious event in human pregnancy frequently leading to brain damage of the fetus or grave complications for the mother. A primate model astonishingly close to the human disease has been developed. A remarkable stability of fetal signs has been found during progressive asphyxia but super-imposed uterine contractions or extreme asphyxia affect the fetal vital signs and warn of impending brain damage or death. Acute fetal asphyxia damages the brain stem early and only much later the basal ganglia or cortex while prolonged partial asphyxia damages cortex, white matter and the basal ganglia and mimics the perinatal brain damage seen in human beings. These studies relate directly to the troublesome dysfunction of brain known to originate during the perinatal period. Another brain study used the superfused cerebral cortex of primates and emphasized problems of trauma and ischemia and the associated edema. The experimental preparations embody features common to the clinical conditions of trauma and stroke and the findings now emerging in relation to permeability of the blood brain barrier (BBB) appear to have relevance for the treatment of brain edema. Further studies on permeability using peroxidase as an enzyme tracer defined a blood nerve barrier (BNB) which creates an endoneurial compartment that allows peripheral nerve to maintain its homeostatic chemical environment. Putting tracers into central nervous systems compartments revealed some basic permeability features of potential significance for understanding certain pathological conditions. In brain tumors induced by virus the peroxidase tracer easily penetrated fenestrated capillaries indicating the lack of a normal blood brain barrier in the presence of tumors.

We continue to recognize that the treatment of hereditary disease will depend ultimately upon more knowledge about genetic control mechanisms in cells. The problem has been approached by the study of cell differentiation in bacterial and other systems with large numbers of replicating cells available for biochemical investigations. Biochemical mutants have been used to analyze why differentiation is suppressed during growth and which biochemical reactions are needed for differentiation. At the beginning of development drastic metabolic changes occur in the production of ATP and other metabolites. The commitments of cells to differentiate correlates with changes in the membrane transport mechanisms. Ribosomal RNA synthesis is preferentially inhibited by guanosine -3' -di-5' - diphosphate. This compound also inhibits other enzymes involved in nucleic acid synthesis indicating control of RNA synthesis during growth and differentiation. Some developmental mutants can be related to human hereditary diseases in that they do not destroy the organism but induce a metabolic change. Attempts are under way to cure such developmental mutants either by the addition of appropriate compounds or by supplying specific DNA. Similar experiments are planned in mutant strains of inbred mice.

Our interest in cerebral trauma from head injury continues and gains extensive attention outside and support from the Department of Transportation. A basis has been established for relating animal experimentation to the human condition.

The importance of rotational displacement of the head in producing brain distortion has been recognized and the rotational frequency of the distortion has been defined. The ability to identify an injury threshold for cerebral concussion provides the possibility of establishing more adequate performance criteria for protection of the brain during head impact and whiplash injury. A finding of practical importance was that a cervical support increases the threshold for experimental concussion. Experimental whiplash produced visible hemorrhages on the brain surface. Postmortem studies on whiplash in human beings support the scaling criteria developed from the experimental design.

Extensive studies on the Dyskinesias have continued to produce significant information. Afferent connections were found between the cortex and those subcortical basal nuclei influenced by the cerebellar nuclei. Other experiments indicated that the activity of some cortical nerve cells depend on the activity of thalamic neurones. A long-term project was completed which defined both the cytoarchitectonics of the human thalamic nuclei and the cerebrothalamic relationships found in a large series of human hemispheres. The variations of thalamic and diencephalic-mesencephalic structures to stereotaxic coordinates have been determined in three planes. This work is basic to location of selective sites in various neurosurgical procedures. The long-term results of unilateral thalotomy in the treatment of Parkinsonism were evaluated in a large group of patients in reference to treatment of the disease with L-dopa. The study indicated that the best initial therapeutic effect may be achieved by a combination of drug and surgical treatment.

We continue to support vigorously research that adds new knowledge on the function of the nerve cell, its membranes and its interaction with other cells. These studies form a rewarding array of diversified investigations ranging from definition of the physical properties of single cell membranes to the development of sensory prosthesis for the blind. The latter devices would operate by electrical stimulation of the visual cortex or other parts of the visual system. Feasibility studies include determining the nature of the visual sensations elicited and the effect of electrode implantation on the brain during prolonged stimulation. Development of a sensory prosthesis necessitates obtaining much new basic knowledge on neural control. Studies are under way on techniques for making connections with the nervous system and on the motor control mechanisms in the cortex and spinal cord. Investigation of the electrophysiological correlates of learned motor acts and the microstimulation of the nervous system also furnish needed information. Neurophysiological studies on photoreceptors propose to establish what mechanisms operate in the transformation of light into neural responses. Many features of the depolarizing generator potentials of photoreceptors have been identified. The continuing research aims to establish how neural interactions contribute to the processing of the information which receptor organs send to the central nervous system. Electronmicroscopical studies on the synaptic organization of the retina are used to relate the neurophysiological function of the synapse to structure. Excitation of the photoreceptors was found to be facilitory to other receptors while excitation of the horizontal cells inhibited large areas of the retina. Other studies on the visual system include an examination of the molecular events leading to excitation of photoreceptors. The development of two new microspectrophotometers permits the studies to be performed in single cells with a rapid time resolution of the chemical response. Studies on the crystalline lens have uncovered chemical agents which influence transparency and proliferation of the lens epithelium. The lesions mimic those associated with diabetes in man and the findings may be useful in understanding the pathology of human cataracts and retinopathies.

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Report of the Acting Clinical Director
National Institute of Neurological Diseases and Stroke

J. M. Van Buren, M. D.

The clinical and clinically applied studies of IR, NINDS fall within the province of the Branches of Electroencephalography, Surgical Neurology and Medical Neurology.

The Branch of Electroencephalography has continued its important service role providing 1582 EEG examinations in the past year, 30% of them to support patient care outside NINDS. Clinical studies in epilepsy, in conjunction with the Branch of Surgical Neurology, have consisted in review of 200 cases of epilepsy with a fronto-centro-parietal localization to determine the localizing significance of various sensory, motor and autonomic aspects of the seizure patterns. Other studies include the effect of temporal lobectomy upon the reaction time and the recovery of this function post surgery. Preparations are being made to study the effect of photically evoked responses in close temporal relationship to spontaneous paroxysmal discharges.

Experimental efforts have centered upon the pathophysiology of penicillin-induced epileptiform activity in the cat hippocampus recorded with intra- and extracellular electrodes. The object has been to define the role of various neurons in production of interictal paroxysmal activity and the transition to rhythmical self sustained ictal activity as well as to define the minimal amount of synaptic activity sufficient for development of these typical epileptiform manifestations. In the hypothalamus the response of the neurosecretory nuclei (paraventricular and supraoptic) to various agents has been investigated. Many of the cells has been shown to have a prominent degree of temperature dependent activity. The activity was depressed by norepinephrine, GABA, glycine, antidromic stimulation of the neurohyphophysis and ADH. The effect of acetylcholine was variable while angiotensin and prostaglandin had an excitatory effect. In several instances opposite effects were seen in cortical and the neurosecretory neurons.

The work in Surgical Neurology is divided into five major programs which involve individual patients in multiple studies and thus make most efficient use of patient care expenditures:

I. Tumors of the CNS: Although experience with the chemotherapy of glioblastoma continues, major emphasis has been placed upon developing baseline indicators of therapeutic response. Using a canine model, studies of radioisotope scanning detection and localization methods have proceeded with a variety of lesions including experimental gliomas. Special interest has been taken in radioactive anti-tumor substances. The tomoscanner has reached a functional level and will be installed in the Clinical Center. Determination of the normal membrane characteristics of CNS cells has opened possibilities of extending these studies to neoplastic cells and their use as an index of response to antimetabolites.

II. Vascular disease of the CNS: Experience is being expanded in the surgical treatment of vascular malformations of the spinal cord and cerebrum and a collaborative study in India is planned to make use of their huge reservoir of material. A search for improvements in spinal angiographic methods continues in the monkey. Studies related to human stroke have included observations of the effects of major venous ligations in monkeys, the differing prognostic importance of intimal versus medial calcification of the human carotid siphon and the efficiency of cerebral arterial crossover in man.

III. Dyskinesias: A long term project, basic to ventriculographic localization, has been finished which defines the cytoarchitecture, cerebral connections and variations of the thalamus and diencephalon in man. This has permitted meaningful studies of unit activity in man and supplements current experimental studies of extrapyramidal and sensory control of motor function. A late (3-10 year) follow-up study of Parkinsonians treated by unilateral thalamotomy with carefully defined lesions and both qualitative and quantitative evaluation of motor function has demonstrated the synergistic role of thalamotomy and L-dopa in the treatment of Parkinsonism.

IV. Effects of trauma on the CNS: Efforts in this area have centered upon the basic definitions of the mechanics of head injury, development of a satisfactory animal model and derivation of the necessary factors to permit scaling to man. This data provides more adequate performance criteria for brain protection during head injury and whiplash. From a practical standpoint a cervical support has been found to increase the threshold for experimental concussion in the monkey.

V. Speech, memory and cognitative function in man: Implanted electrodes and craniotomies with local anesthesia have permitted a variety of studies of higher function in dominant and non-dominant temporo-parietal regions; the amygdala, hippocampus and cingulum. Particular attention has been paid to use of quantifiable techniques and close statistical control. One interesting finding has been that cerebral potentials evoked by tachistoscopic presentation of meaningful and nonsense material appear to differ and provide clues as to the learning process.

The Section on Child Neurology forms an independent unit in SN. In the area of metabolic diseases of the CNS differential features of the cortical degeneration of the Hunter's and Hurler's patients as opposed to Sanfilippo's type of mucopolysaccharidosis have served to explain some differing clinical features of the diseases. In late infantile G_{M1}-gangliosidosis the presence of decreased β-galactosidase activity in the white cells of heterozygous carriers will permit premarital counseling of siblings and relatives. Experimental studies to elucidate the mechanism of ketogenic diet in epilepsy showed an increased content of cholesterol and phospholipids in the synaptic vesicles and nerve endings of weanling rats fed high fat diets. This finding may be related to the increased threshold of excitability. Lastly, a comprehensive study, "Malformations of the Nervous System: Medical Aspects" was finished which provides clues regarding etiology and pathogenesis and forms a guide to the long range management of some of these conditions.

The Branch of Medical Neurology presents an inter-related multidimensional attack on the chosen target diseases emphasized in the application of basic research techniques to clinical neurologic problems, especially utilizing tissue and blood specimens obtained from patients. The techniques consist of: histochemistry, clinical neurophysiology, biochemistry, immunology, electron-microscopy, and microbiology (including tissue culture). In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, sub-forms; (c) specific or symptomatic therapy; and (d) animal models closely related to the human pathophysiologic states.

Major accomplishments include: (a) a new hypothesis on the pathogenesis of myasthenia gravis and a new and very effective treatment (high-single-dose alternate-day prednisone) thereof; (b) further animal-model support for our hypothesis that the pathogenesis of the Duchenne form of muscular dystrophy is based on a vascular abnormality of skeletal muscle; (c) published effectiveness of a new treatment of hypokalemic periodic paralysis, with acetazolamide; (d) a new hypothesis on the basic role of qualitative and/or quantitative abnormality of the motor neuron in several disorders generally thought to be "myopathic," e.g., myotonic dystrophy, corticosteroid atrophy, and cachectic atrophy associated with cancer; (e) a new open-biopsy electromyography (EMG) technqiue for obtaining absolute EMG-histochemical correlation of normal and abnormal human muscle; (f) a method of obtaining reproducible tissue culture growth of human muscle fibers in 75% of explants from open or needle biopsies of children and adults.

For the clinical investigations, 292 patients were admitted for a total of 5,197 patient days, and there were 517 outpatient visits. There were 223 muscle, nerve and brain biopsies obtained. The neurologists provided consultation on 402 patients of other departments. The one-year approved residency training program in neurology has continued.

Serial No. NDS(I)-66-ODIR 1460
1. Office of the Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Speech habilitation of the congenitally deaf--
Fundamental problems and suggested directions

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: None

Man Years:	
Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To analyze the reasons for the general lack of success in speech habilitation of the congenitally deaf and to suggest areas of research which might contribute to progress.

Methods:

1. Review of literature.

2. Consultation with teachers of the deaf, and experts in allied fields-- engineering, speech studies, and physiology, among others.

3. Analysis of problems.

Major Findings: One of the major issues in habilitation of the congenitally deaf is the effect of very early intervention, i.e., the use of hearing aids, and speech and language training from early infancy. In an attempt to gather objective evidence demonstrating the benefits of such programs, letters of inquiry were sent to the outstanding school for the deaf, both here and abroad. These included the Audiology Department of the University of Manchester, Johns Hopkins, University of Kansas, Clarke School for the Deaf, St. Joseph's Hospital, Dr. B.T. Tervoort

(Netherlands), Bill Wilkerson Clinic, and others. The replies were almost uniformly negative; practically no such data exists. An attempt was launched with Dr. Lois Elliott in the Office of Education, to set up a program to gather such data. Lack of support at NIH has suspended participation in this work.

Proposed Course of Project: To follow developments in the field and possibly resume active work at some later date.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68-ODIR 1554
1. Office of the Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pure and applied mathematics, with special attention to mathematical models of sensory systems

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: Mathematical Research Branch, NIAMD

Man Years:
Total: 0.1
Professional: 0.1
Others: 0.1

Project Description:

Objectives: To conduct research in pure and applied mathematics, with special attention to mathematical models of sensory systems.

Methods Employed: Mathematical Analysis

Major Findings: Graph and matrix theory - an equation relating to coefficients of the characteristic equation to the traces of powers of the matrix and the cyclic structure of the graph of the matrix were further developed.

Proposed Course of Project: To continue at a low level in the fields of visual models and the mathematics of graphs and matrices.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70-ODIR 1789
1. Office of the Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The physical senses

Previous Serial Number: Same

Principal Investigator: Edith L.R. Corliss, Physicist

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.3

Project Description:

Objectives: Preparation of a publication summarizing and interpreting experimental results on the properties of the normal human senses of sight, touch and hearing. These intact human senses are to be considered quantitatively somewhat by analogy to the description and evaluation of measuring instruments, i.e., which mechanisms are optimized, what resolution limits are obtained, over what dynamic range do they function?

Major Findings: Library research has continued into current experimental data on the performance characteristics of the senses of sight, touch and hearing. Completed at least as far as first drafts: Introduction, Preface, "The Ear as a Mechanism of Communication" (comments received and some more revisions made). Nearly completed, but not yet circulated for comment: "The Ear as a Measuring Instrument", "The Ear as a Communication System" (recognition of speech, pitch, and rhythm).

Sections of "The Ear as a Mechanism of Communication" and "The Ear as a Communication System" have been assembled as a general article for publication in a scientific journal, under the title "The Recognition of Speech". Other sections of these chapters, relating to those functions of the ear that can be represented by a model using synchronous firings and an AND gate, are being summarized and with additional new material, prepared as a paper describing the behavior of the ear as a coincidence-counting device, under the tentative title (if permitted by the journal's editorial policy) of "Purely by Coincidence". Although it is not yet verified, it appears that this type of function, if in fact performed by the ear, might also provide a rigorous model for the subjective observation that some features of pitch recur in every octave change in frequency. At present this empirically known phenomenon is not explained by a general theory.

Proposed Course of Project: Two more papers can be assembled from the materials now written, namely "The Perception of Sound Intensity" (a systematic review of our experimental knowledge of loudness) and "Review of Concepts of the 'Critical Band'".

Preparation of independent, free-standing papers requires added time and effort. Two desirable side effects are that the material is revised with added editorial review, and that some practical and useful results are produced well in advance of the completion of the remainder of the work. It should become clear when the sections on hearing are complete whether these subsidiary efforts produce significant improvements in the final draft. As before, chapters of the book will be circulated for comment by other investigators.

Honors and Awards: None

Publications: Scheduled for the August 1971 issue of the Journal of the Acoustical Society of America: "Estimate of the Inherent Channel Capacity of the Human Ear". (This work, sponsored by the National Bureau of Standards, is the background material for the latter half of the chapter on "The Ear as a Mechanism of Communication").

Serial No. NDS(I)-70-ODIR 1790
1. Office of Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structure and function in the first synaptic layer of dogfish shark retina

Previous Serial Number: Same

Principal Investigator: Henry G. Wagner, M.D.

Other Investigators: William K. Stell, Ph.D., M.D.
Akimichi Kaneko, M.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years:

Total:	0.75
Professional:	0.75
Other:	0.0

Project Description:

Objectives: To examine the structural and functional relations of horizontal cells and other elements in the distal regions of the retina of the dogfish, *Mustelus canis*.

Methods Employed:

1. Electrophysiology: The isolated dogfish retina was penetrated with three ultrafine micropipettes, two filled with Procion dyes for recording and making location of the units, and one filled with potassium chloride, for passing current intracellularly. Marked retinas were processed for localization of cells in the fluorescence microscope.
2. Retinas of dogfish and other sharks were fixed, embedded and examined by conventional techniques, including staining *en bloc* with uranyl acetate to demonstrate "gap" junctions.

Major Findings:

1. Horizontal cells of the first row are electrically coupled to one another but not to horizontal cells of the second

row or to bipolar cells. Coupling may be detected in cells separated by as many as 6 cell diameters.

2. Preliminary ultrastructural study indicates that horizontal cells of the first row in sharks are joined by gap junctions to one another but not to cells of other types.

Significance: Studies reported in the literature show that horizontal cells of the same row are joined by some kind of apposition in all fishes. Pending results of further studies, it may be assumed that these appositions are gap junctions which mediate electrical coupling between cells, as they have been shown to do in numerous instances. Such coupling may account for the immense area effect (horizontal spread) of S-potentials (horizontal cell responses) in fish retinas. The role of this extensive spatial summation, and of the horizontal cells themselves, remains obscure.

Proposed Course of Project: Further studies are needed on the functional and structural interrelations of horizontal cells and other retinal elements. These will be pursued in subsequent summers at the Marine Biological Laboratory. A report of the electrophysiological data has been prepared and submitted for publication by Dr. Kaneko. Project completed.

Honors and Awards: None

Publications: KANEKO, A.: Electrical connections between horizontal cells in dogfish retina. J. Physiol. 213: 95-105, 1971

Serial No. NDS(I)-70-ODIR 1860
1. Office of Director of
   Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The morphological organization of the vertebrate retina

Previous Serial Number: Same

Principal Investigator: William K. Stell, Ph.D., M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.6
Other: 0.0

Project Description:

Objectives: To prepare a comprehensive critical review of scientific literature concerning the structural, ultrastructural, functional and chemical subunits of the vertebrate retina and their interconnections. Particular attention is devoted to recent publications concerning synaptic ultrastructure, histochemical localization of presumed neurotransmitters, and histological localization of responding cells.

Findings: A comprehensive review of retinal organization was prepared.

Significance: This is the first review in seven years in a very rapidly advancing field and should be a useful reference work for investigators concerned with structure and function in the vertebrate retina. It will be published in the Handbook of Sensory Physiology, Volume VII/Part 1.B. Physiology of Photoreceptor Organs. Edited by M.G.F. Fuortes. Berlin, Heidelberg, and New York: Springer Verlag (1971).

Proposed Course of Project: Project completed.

Honors and Awards: None

Publications:

Stell, W.K.: The morphological organization of the vertebrate retina. The Handbook of Sensory Physiology, Volume VII/Part 1.B. Physiology of Photoreceptor Organs. Edited by M.G.F. Fuortes. Berlin, Heidelberg, and New York: Springer Verlag. (In press).

Serial No. NDS(I)-70-ODIR 1861
1. Office of Director
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Synaptic connections in the first synaptic layer in the retina of a marine teleost, *Eugerres plumieri*

Previous Serial Number: Same

Principal Investigator: Miguel Laufer, Ph.D., M.D.

Other Investigators: William K. Stell, Ph.D., M.D.

Cooperating Institution: Departamento de Neurobiologia, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela

Man Years:
Total: 0.0
Professional: 0.0
Other: 0.0

Project Terminated.

Serial No. NDS(I)-70-ODIR 1862
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The morphology of photoreceptors in the ventral eye of the horseshoe crab, Limulus polyphemus

Previous Serial Number: Same

Principal Investigator: William K. Stell, Ph.D., M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.7
Other: 0.0

Project Description:

Objectives: To study the structure and ultrastructure of photoreceptor cells in the ventral eye of Limulus.

Methods Employed: Ventral eyes of adult horseshoe crabs were dissected free end fixed in appropriate agents after incubation in one of the following:

1. Artificial sea water (ASW) at 20°C.
2. ASW lacking Na^+, K^+ or Ca^{++}.
3. ASW continuing ouabain or strophanthidin.
4. ASW at 4°C.
5. ASW containing horseradish peroxidase or microperoxidase.

Fixed nerves were embedded in epoxy resins and either sectioned serially at 1.0-2.5μm for light microscopy or thin-sectioned for electron microscopy.

Major Findings:

1. The ventral eye of Limulus contains photoreceptor cells of two types, large (about 100x200 μm) and small (about 50x100 μm). Both are enclosed in many layers of glial cells, have large (15-20 μm diamter) axons, and have a specialized photoreceptive surface called the rhabdomere. Small cells are joined together

along patches of rhabdomere and all their rhabdomere is located at such junctions; these cells are joined this way in clusters of 3-9. Large cells also have rhabdomere at junctions which they make infrequently with other large cells or small cells. Most of the large cells are isolated however, and even when they are coupled, most of their rhabdomere is distributed away from the junctional region.

2. Both large and small cells are contacted on or near the rhabdomere by fine axons containing small vesicles and larger granular inclusions.

3. The rhabdomere of large or small cells which have been incubated in artificial sea warer at 20°C. for at least 30 minutes comprises many closely-packed microvilli. If fixed within minutes after removal from the animal however, the rhabdomere may instead consist of fin-like projections, folded over one another to present complex lamellar or whorled configurations. The configurations were seen only in some large cells and never in small cells.

4. Regardless of whether the rhabdomeric membranes form whorls or microvilli, they normally appear to be closely apposed, separated by a space no wider than 5-10 Å. Since this "space" is penetrated readily by microperoxidase (molecular weight ca. 2000) but apparently not by horseradish peroxidase (m.w. 40,000), the closeness of membrane apposition observed in fixed material may correspond closely to that in the living state.

5. Incubation in the cold, in sea water containing ouabain or strophanthidin, or in Na^+ -or K^+ -free solution causes the microvilli to appear separated by a much larger space (ca. 100 Å). The effect of Na^+-free treatment, at least, is reversed by returning the cells to Na^+ -containing solution.

6. Ten minutes adaptation to darkness or bright light has no noticeable effect on rhabdomere structure.

7. The channels joining the extracellular space to the axonal membrane, unlike those joining it to rhabdomeric membranes, are patent to horseradish peroxidase.

Significance: The significance of differences between large and small cells is not known. Probably only the former have been studied well by electrophysiological methods, which favor stable penetration only of larger cells. Since the rhabdomeric junctions between cells (especially the small ones) appear identical to those between retinula cells in the lateral eye of *Limulus*, the ventral eye cells are probably likewise electrically coupled. Experiments are in progress to determine whether the whorly rhabdomere observed in freshly dissected preparations represents accurately the state *in vivo* or is brought about by manipulation during dissection. The separation of rhabdomeric membranes by procedures which inhibit the sodium pump (cold, Na^+ - and K^+ -depletion, ouabain and strophanthidin) and inhibit the light response is consistent with the conclusion that the close appositions are maintained by pump activity or instrumental in the response mechanism, but may be explained also by changes in ionic distribution or osmotic properties of membranes. Since there appears to be no barrier to the passage even of large protein molecules between the axonal membrane and extracellular space, the glial sheath does not appear to contribute to a high space constant which might be expected in these axons, since they are not known to generate propagated nerve impulses. The presence of small axon terminals on or near the rhabdomere suggests a system for control of the receptor response at its site of origin.

Proposed Course of Project: The project is to be completed and the findings published as soon as possible, pending progress of correlated electrophysiological studies.

Honors and Awards: None

Publications:

Stell, W.K., and Ravitz, M.J.: The structure of neurons in the ventral photoreceptor organ of the horseshoe crab, *Limulus polyphemus*. *J. Cell Biol*. 47: 202a-203a, 1970

Serial No. NDS(I)-71-ODIR 1863
1. Office of Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structure and relationships of cells in the retina of the smooth dogfish, Mustelus Canis.

Previous Serial Number: None

Principal Investigator: William K. Stell, Ph.D., M.D.

Other Investigators: Paul Witkovsky, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years:
Total: 1.8
Professional: 1.2
Other: 0.6

Project Description:

Objectives: To describe in detail by light and electron microscopy the form and relationships of neurons in the retina of the smooth dogfish, Mustelus. This study is part of a larger effort, including electrophysiological investigations by Drs. Stell, Wagner and Wolbarsht (q.v.), aimed at analysing the structure and function of synaptic networks in a primitive but complex retina.

Methods Employed: Flat-mounted and sectioned retinas stained with methylene blue (intra vitam), reduced silver, or the Golgi method; routine ultrastructure and reconstruction from serial ultrathin sections.

Major Findings:

1. The dogfish retina is highly stratified. Distinct morphological types of neurons, and in at least some instances the synapses between them, are segregated more or less into different retinal sublayers.

2. Some horizontal, bipolar, and ganglion cells are exceptionally large, and therefore probably favorable for

penetration by micropipette electrodes.

3. There are at least four or five varieties of bipolar cells, each characteristic in the size, form, and location of its dendritic tree, cell body, axon and telodendron. Bipolars of some types have characteristic "Landolt's clubs" while others do not.

4. Giant ganglion cells with dendritic fields about 2 mm in diameter have been observed in three sublayers of the inner plexiform layer. The giant cells of the proximal (conventional ganglion cell) layer are so arranged that in general the dendrites of one cell just reach to the perikaryon of the 5 or 6 nearest giant cells -- i.e. they form an approximately hexagonal mosaic with average cell-cell separations of about 1 mm.

5. A quantitative morphological description is being undertaken which will facilitate treatment of these cells by a Rall-type neuron model.

6. Synaptic endings of bipolar cells and their relationships have been reconstructed from serial ultrathin sections. Most (72%) of the processes contacting these endings appear to be "axonal" (probably amacrine cells) inputs; of these, about 1/3 also receive a reciprocal input from the bipolar cell at a ribbon contact. The remaining processes (28%), probably mainly ganglion cell dendrites, only receive a ribbon synapse. The several types of terminals examined to date appear not to differ greatly in these proportions.

Significance: Little is known of the detailed synaptology in a retina which is at the same time so primitive and so complex. Particularly attractive is the prospect of obtaining correspondingly detailed functional data on bipolar and ganglion cell circuitry (Wagner, Stell and Wolbarsht).

Proposed Course of Project: More histological preparations will be made in order to study amacrine cells and the remainder of the ganglion cell population. Electron microscopy will be used to investigate connections of cells of identified subvarieties in further detail.

Honors and Awards: None

Publications:

Witkovsky, P., and Stell, W.K.: Gross morphology and synaptic relationships of bipolar cells in the retina of the smooth dogfish, *Mustelus Canis*. *Anat. Rec.* 169: 456-457, 1971

Serial No. NDS(I)-71-ODIR 1864
1. Office of Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Receptive field organization of ganglion cells in the retina of the smooth dogfish, Mustelus Canis.

Previous Serial Number: None

Principal Investigator: Henry G. Wagner, M.D.

Other Investigators: William K. Stell, Ph.D., M.D.
Myron L. Wolbarsht, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To investigate the synaptic organization of the dogfish retina by means of the spatial, temporal, and chromatic properties of ganglion cells.

Methods Employed: Pieces of isolated eye cup (retina attached) at about 20-22°C. were suffused with moist 100% oxygen and presented patterned monochromatic light stimuli through a dual-beam optical system incorporating a grating monochromator. Action potentials were recorded extracellularly with class-coated Pt-Ir microelectrodes inserted through the vitreous body, amplified and displayed conventionally.

Major Findings: About two-thirds of the units isolated responded at threshold to a small centered spot of any wavelength with an ON-response, after a latent period of about 0.5 second. These responses summated linearly for spot diameters up to 1-2 mm. With larger spots, summation failed and inhibition was usually observed. Annuli of inner diameter greater than

1-2 mm presented simultaneously with a centrally placed stimulus spot elevated the threshold for the ON-response. Such annuli inhibited spontaneous dark activity which was present infrequently, but annuli alone evoked no response. The excitatory and inhibitory regions were concentric and roughly circular. The inhibitory region was observed to extend from a diameter of 2.0 to 5.0-10.0 mm but its full central and peripheral extent was not determined. The spectral sensitivity of both the excitatory and inhibitory processes was identical; it was maximal at 500-510 nm but the function appeared narrower than expected from corresponding pigment spectra, perhaps because of preferential reflection of light of certain wavelengths by the choroidal tapetum lucidum.

Significance: Ganglion cells with complex receptive fields several millimeters in diameter have been described in several vertebrates, but in none were the receptive field centers as large as 2 mm. The size and simplicity of these units renders them very convenient for investigating spatial interactions in the retina. Correlative studies of neuronal structure are being conducted by Dr. Stell in collaboration with Dr. Paul Witkovsky of Columbia University College of Physicians and Surgeons.

Proposed Course of Project: Further studies of these giant ganglion cells at the Marine Biological Laboratory during the summer of 1971 will include: attempts to determine the full central and peripheral extent of the inhibitory field (surround); examination of suprathreshold properties; histological identification of ganglion cells having large fields; and the activity of ganglion cells during intracellular recording and electrical polarization of identified bipolar cells. Ganglion cells with other types of receptive fields also will be sought.

Honors and Awards: None

Publications:

Stell, W.K., Wagner, H.G., and Wolbarsht, M.L: Receptive field organization of ganglion cells in the retina of the smooth dogfish, Mustelus Canis. Biol. Bull. 139: 437-438, 1970

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological Diseases and Stroke

July 1, 1970 - June 30, 1971

James S. Bryan, Chief

During the past 12 months, the Section on Technical Development continued to fulfill its traditional role of support of NIMH and NINDS research efforts. Continuing assistance was made available in shop help, special purpose instruments, instrumentation systems, and assistance in computer oriented research and digital instrumentation.

The major load on the Section in the past year has been the check-out, acceptance and custom interface fabrication associated with the NIMH Building 10 on-line computer system (Systems Engineering Laboratories 810B) which was installed July 23, 1970 and accepted on August 23, 1970. Hardware design and fabrication for the system, although far from complete, is now beginning to taper off and the major remaining effort will lie in the area of software support for new users. Usage of this equipment has thus far been limited to software development and a few small batch applications. Scheduled on-line use is expected to begin in May 1971.

Appendix II shows a breakdown by Laboratory of the usage of the three small laboratory-type computers maintained and supported by the Section. All of this equipment is now loaded near capacity.

Staff changes involved the addition of one electronic technician, a systems programmer for the 810B and the loss of our two part-time student assistants.

Appendix I shows a breakdown of section activity by laboratory. As usual, almost all research within the section has involved collaboration with laboratories in NIMH and NINDS and is, therefore, reported as part of the projects in the laboratories involved. Exceptions to the foregoing include a small but continuing effort in neural modeling, and development of equipment to investigate the nature of motor deficits in patients with dystonia or Parkinson's disease.

Our most recent neural modeling effort has involved the development in collaboration with the Laboratory of Applied Studies, DCRT, of a model neuron with a multi-compartment dendritic network capable of simulating nonlinear spatial summation. Future work will involve the application of this neuron in collaboration with the Laboratory of Neurophysiology, NINDS, to model the responses of ganglion cells in turtle retina. The intent is to define the role of the horizontal cells in vision.

A total of 199 projects were completed in the last year. A large portion of these consisted of routine fabrication of various chambers, baths, electrode holders, and jigs for experimental use as well as many small electronic devices.

Major electronic systems included:

(1) An automatic antenna switching system for use in small animal EEG telemetry in the 100x100 ft. enclosure at the National Zoological Park. - Laboratory of Psychobiology, NIMH.

(2) An improved variable amplitude, variable frequency oscillator with low harmonic content for use as a stimulus generator in auditory work. - Behavioral Biology Branch, NICHD.

(3) A system to achieve programmed gating of speech from tape recorded utterances. - Laboratory of Psychology, NIMH.

(4) Expansion of the rat-colony data acquisition system at Poolesville. - Laboratory of Psychology, NIMH.

(5) A device to extract the logarithm of interspike intervals from single neuron activity. - Laboratory of Neurophysiology, NINDS

(6) A device to verify calibration of EEG equipment while in use by means of inductive coupling to patient leads. - Office of the Director, NINDS.

APPENDIX I

Apportionment of Effort

ENGINEERING AND FABRICATION TIME

LABORATORY OR BRANCH	HOURS	PERCENT
NIMH Computer, IR, DCBR, NIMH	8757	31.63
Psychology, NIMH	4885	17.64
Neurophysiology, NINDS	1911	6.90
Behavioral Biology, NICHD	1779	6.43
Biophysics, NINDS	1708	6.17
Clinical Psychobiology, NIMH	1387	5.01
Technical Development, NIMH-NINDS	1142	4.12
Office of the Director, NINDS	1060	3.83
Electroencephalography, NINDS	896	3.24
Neurophysiology, NIMH	762	2.75
Adult Psychiatry, NIMH	736	2.65
Child Research, NIMH	647	2.34
Experimental Neurology, NINDS	448	1.61
Division of Special Mental Health Research, NIMH	260	.94
Clinical Science, NIMH	222	.80
Medical Neurology, NINDS	204	.74
Cerebral Metabolism, NIMH	182	.65
Neuropathology & Neuroanatomical Sciences, NINDS	157	.57
Neural Control, NINDS	145	.52
Neurochemistry, NINDS	110	.40
All other Labs	288	1.04
NIMH (Total)	18,014	67.9
NINDS (Total)	6,751	25.4
NICHD (Total)	1,779	6.7
TOTAL*	26,544	100.0

*Not included in the foregoing totals or in the breakdown by laboratory are 1982 hours spent in routine and computer maintenance internal to the Section, and the time of the Section Chief. Totals and percentage figures do not include time charged to the Section on Technical Development.

APPENDIX II

Laboratory Computer Utilization

Classic LINC	2996 hours
MicroLINC	2060 hours
PDP-12	1770 hours

LABORATORY OR BRANCH	LINC %	µLINC %	PDP-12 %	Total %
Overhead	.5	6.0	2.0	2.5
Psychology, NIMH	45.5	--	10.0	23.0
Technical Development, NIMH	--	3.0	4.5	2.0
Adult Psychiatry, NIMH	45.0	1.0	13.0	23.0
Socio-environmental Studies,NIMH	2.0	--	--	1.0
Neurophysiology, NIMH	--	40.0	3.0	13.0
Clinical Psychobiology, NIMH	3.0	--	0.5	1.5
Neural Control, NINDS	--	8.0	3.0	3.5
Biophysics, NINDS	--	25.0	3.0	8.0
Surgical Neurology, NINDS	4.0	--	--	1.5
Neurophysiology, NINDS	--	--	43.5	11.0
Electroencephalography, NINDS	--	--	3.5	1.0
Collaborative & Field Research,NINDS	--	--	13.0	3.5
Behavioral Biology, NICHD	--	17.0	.5	5.5
Physical Biology, NIAMD	--	--	.5	--
Total NIMH	96.0	46.5	32.0	65.0
Total NINDS	4.0	35.0	67.5	29.5
Total NICHD	--	18.5	0.5	5.5

Annual Report
July 1, 1970 through June 30, 1971
Medical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, clinical neurophysiology, biochemistry, immunology, electron-microscopy, and microbiology (including tissue culture). In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new, sub-forms; (c) specific or symptomatic therapy; and (d) animal models closely related to the human pathophysiologic states.

For the clinical investigations, 292 patients were admitted for a total of 5,197 patient days, and there were 517 outpatient visits. There were 223 muscle, nerve and brain biopsies. The neurologists provided consultation on 402 patients of other departments in the Clinical Center, performing indicated myelograms, pneumoencephalograms, and cerebral angiograms.

The one-year approved residency training program in neurology has continued. Fifteen neurologists and other physicians and 11 technicians came this past year as guest workers to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry thereto. An audio-visual self-teaching carrel has been developed, for which programs on general and specific topics of clinical research in neuromuscular disorders are being assembled.

The Department of Neurology, Warsaw Medical Academy is continuing its vigorous collaborative research program in neuromuscular disease with us under the PL-480 program.

Muscular Dystrophies and Other Non-Inflammatory Myopathies: A new hypothesis, based on muscle histochemical studies, was reported concerning the pathogenesis of progressive pseudohypertrophic muscular dystrophy of Duchenne. Because the earliest histochemical change is a grouped pattern of degenerating and regenerating muscle fibers, it was proposed that the disease is caused by an abnormality of small arterial blood vessels on either a structural or functional basis. That principle was established and the hypothesis shown to be possible by the production of identical muscle lesions early after embolization of rabbit arteries (structural arterial occulusion) with dextran particles; and repeated embolization over 3 months reproduced the more chronic changes of Duchenne dystrophy. It was pointed out that the subtle, histochemically defined abnormalities in clinically normal carriers of Duchenne dystrophy, namely 1-3 fibers together undergoing necrosis and/or regeneration, are also in accord with this hypothesis and they too were reproduced in the minimally affected regions following embolization. We have now produced the same early and late Duchenne-like muscle lesions with non-structural, presumably functional, lesions of intramuscular blood vessels by combining a subthreshold vascular factor (aortic ligation) and a subthreshold dose of a vasoactive amine (either serotonin or norephinepherin), thereby establishing the possibility of a functional malfunction of blood vessels being the basis of Duchenne dystrophy. Defects of blood vessels and/or vasoactive amines are now being sought in the patients.

From a correlative study of regenerating muscle fibers following experimental injury in animals, it was reported that ultrastructural changes are predictable from histochemical findings. In general, mitochondria were much more susceptible than myofibrils, the reverse of what is found in denervation atrophy.

In progressive external ophthalmyoplegia it was found that "ragged red fibers" are present in clinically normal limb muscle (as well as weak muscle) and are characteristic of the syndrome. Such fibers have excessive and morphologically bizarre mitochondria and excessive lipid droplets.

Tubular aggregates were reported in humans to be unique hyperplasia of the sarcoplasmic reticulum (SR) (longitudinal component and/or lateral sac) restricted to type II muscle fibers. They contain rich DPNH and TPNH tetrazolium reductase activities attributable to "microsomal" DPNH and TPNH cytochrome c reductases. It was postulated that these SR enzymes may help supply energy directly for SR ATP synthesis to drive the SR calcium pump.

For several of the erstwhile "myopathic" disorders, both long-known ones, such as myotonic atrophy (myotonic dystrophy), and recently found ones, such as type I hypotrophy with central nuclei, a new hypothesis concerning their possibly being caused by a lower motor neuron abnormality has been proposed (see"Lower Motor Neuron Disorders").

Inflammatory Myopathies (Idiopathic) - Polymyositis/Dermatomyositis: This disease complex, when beginning over the age of 40, especially in males, is commonly associated with, and perhaps due to, a small distant cancer. The childhood form is not associated with cancer, but both age groups might have similar pathogenic mechanisms. The method of treatment we have introduced, high-single-dose alternate-day glucocorticoid (prednisone), has now proven in our hands to be the best available both for children and adults. It is easier to manage, has greater therapeutic benefit, and fewer side-effects. Our experience extends over 3 1/2 years and includes 20 carefully studied patients. We have proposed a new hypothesis on the mechanism of muscle damage in the polymyositis/dermatomyositis (PM/DM) complex on the basis of our finding deposits of immunoglobulin G, immunoglobulin M, and β_{1C} globulin (C3), alone or in combination, in skeletal muscle blood vessels of 15 of 36 cases of PM/DM, especially in childhood dermatomyositis. We suggest that one of the mechanisms leading to muscle damage in inflammatory myopathy may be vascular abnormality mediated through deposits of immunoglobulin and C3, possibly in the form of immune complexes. This supports our earlier hypothesis that PM/DM is another form of blood-vessel abnormality of muscle. The immunological abnormality could in turn be caused by a viral infection and/or an associated cancer.

hypotrophy with central nuclei and central core disease, and possibly neurogenic (perhaps only in a small part) are benign congenital hypotonia, the facilitating myasthenic syndrome, some cases of facioscapulohumeral disease, and late-onset and congenital rod disease. From such proposals a new classification of neuromuscular disorders has been developed.

The generalized muscle cachectic atrophy and weakness which is a common and often the most crippling manifestation of cancer, as well as of hypercorticosteroidism (Cushing's syndrome and idiotrophic), and disuse, is selective involvement of histochemical type II muscle fibers. We have postulated this to be a pan-neuropathy of all lower motor neurons to which the type II muscle fibers are more susceptible, not a myopathy as is usually stated. This proposal was supported by our report that the ultrastructure aspects of type II atrophy are qualitatively indistinguishable from typical denervation atrophy, but milder in degree. We will next turn our attention to ways to prevent or treat type II atrophy.

Central Nervous System Disorders:

Brain Tumors - An improved method of concentrating and purifying Schmidt-Ruppin Rous avian sarcoma virus (SR-RSV) yielded, 4 weeks after intracerebral inoculation into newborn dogs and hamsters, 96% survivor incidence and 100% brain tumor incidence in survivors, compared to 49% and 86% respectively with previous methods. A quantitative dose-response relationship was demonstrated. The tumors were sarcomas and gliomas (spongioblastomas, astrocytomas, and ependymomas) with varying degrees of anaplasia. Injection of virus near the cerebral surface or in the cerebellum led to sarcomas and deep cerebral periventricular injection to gliomas. No evidence of virus was found in dog brain tumors with electronmicroscopy or immunologic methods, but the SR-RSV genome was detected by inoculating whole cell suspensions of dog and hamster brain sarcomas and gliomas back into avian hosts, wherein typical Rous sarcomas containing C-type viral particles, group specific viral antigens, and infectious RSV were induced. The ultrastructural details of cell types composing these brain tumors have been described. These induced brain tumors are an especially suitable system in which to study mechanisms that allow malignant transformation of glial and mesodermal brain cells by RNA-C-type viruses without detectable evidence of viral replication, as is likely to be occurring in at least some spontaneous human brain tumors. This system will serve as a guide to isolation of viruses causing human brain tumors. The reproducibility and rapidity of experimental tumor induction, type of blood supply, and histologic similarity of the experimental gliomas to spontaneous human gliomas make these SR-RSV-induced animal brain tumors ideal for morphologic, chemotherapeutic and brain-scanning studies. They are now being used to study (with LNNS, IR) the blood-brain-barrier changes in tumors, combining tracer substances with electronmicroscopy.

Epilepsy - The simpler synaptic structure of the hippocampus makes it more suitable for study of epileptic phenomena than neocortex. A powerful recurrent inhibitory system in the hippocampus is known to limit interictal epileptic discharges temporally and spatially. The synaptic input on neuronal somas and large dendrites of the CA_4 area of the cat hippocampus, a key region, is being studied electronmicroscopically to provide a morphological basis for structure-function correlations.

In a family showing hereditary myoclonus epilepsy, a concomitant myopathy with severe morphologic and biochemical (defective NADH oxidation) abnormalities of mitochondria was found. Assuming the same metabolic defect may exist in brain causing the epilepsy, skeletal muscle provides much more accessable tissue for its elucidation. A new syndrome of epilepsy with acanthocytosis, neuromuscular disease, and normal β-lipoprotein has been delineated in a third family.

Spinocerebellar ataxias - One patient with intermittant cerebellar ataxia, who was found to have pyruvate decarboxylase deficiency, had excessive lipid droplets in his skeletal muscle fibers, as did his clinically normal father. Biochemical abnormalities in other types of spinocerebellar ataxia are noted in "Methodology". The various manifestations of the immunologic deficiency state and other metabolic abnormalities in ataxia teleangectasia, found by us over the past 3 years, have been detailed in a chapter.

Psychosis - In acute psychoses, mostly schizophrenic, a subclinical myopathic degeneration of muscle fibers, found with histochemical techniques, was reported in 22 of 52 patients. It is approximately correlated with peak elevations of the muscle-isozyme of creatine phosphokinase in serum of the male but not female patients. This evidence of an extracerebral component suggests the psychosis may be part of a more generalized metabolic disorder (with Dept. of Psychiatry, U. of Chicago).

Other Degenerations - In subacute sclerosing panencephalitis two treatment attempts using transfer factor from the patient's twin were not successful (with PR-CFR).

Methodology and Basic Cellular Mechanisms: Four new aspects of clinical electromyography (EMG) have been developed, facilitated by high-speed tape recording: (a) A new descriptive term for the pattern of short duration, small amplitude, excessively abundant potentials for a given amount of voluntary effort, i.e., "SSAP", was introduced to replace the generally used term "myopathic", a designation which we consider to be misleading at times. We consider SSAPs just as likely to be due to neuronal disease as to myopathic disease. (b) The technique of "open biopsy electromyography" was introduced to provide tight correlation of histochemistry done on the same muscle fibers studied electrically immediately before tissue excision. (c) Using this technique in patients with biopsies showing predominance of either type I or type II muscle fibers, we have identified the different physiological properties of these two human motor unit types. Type I motor units fire with initial effort and discharge more regularly and more continuously, while type II units fire only irregularly in unsustained bursts and only when more vigorous contraction is exerted. (d) The electromyographic patterns of selective type II and type I muscle fiber atrophy in humans have been identified, and an hypothesis developed that relates the electrical data to the neurogenic origin we propose for these atrophies.

Tissue culture of muscle and neurons has been re-established in the Branch. In addition to various studies of cultured muscle and neurons of animals, we have developed a method for obtaining consistent growth in vitro of 75% of the explants from human skeletal muscle taken by open or needle

Rats made severely choline-deficient maintained an essentially normal ability to transmit signals from motor nerve to muscle, even though severe abnormalities were present in other tissues.

In a study of cortical representation of sensation in the monkey, it was concluded that neither SI nor SII is of major importance for fine discriminations of tactile, vibratory, proprioceptive, and two-point sensation, contrary to existing neurophysiological theories (with NIMH).

More precise evaluation of contractile rise time and a derived value for its temperature coefficient were accomplished because a technique and apparatus were developed to effectively eliminate the series elastic component from muscle contractile responses, allowing more accurate study of contraction per se. In a variety of commonly used laboratory mammals, a correlation between whole muscle intrinsic shortening velocity and maximal rate of isometric tension development has been demonstrated.

The responses of intestinal smooth muscle to drugs or ionic changes were studied as a simplified model system for understanding muscle phenomena in general and for its possible relation to vascular smooth muscle in skeletal muscle and brain.

Snake venoms and antivenoms have been furnished to NIH for DBS through a PL-480 collaborative project with Ein Shams University, Cairo, Egypt.

biopsy from children and adults. The patterns of outgrowth have been compared and contrasted with animal muscle growth. Histochemical and electronmicroscopic studies of the cultured normal human muscle are nearly completed. Several types of pathologic human muscle are being grown and the cultures analyzed histochemically and electronmicroscopically.

On the basis of a number of histochemical stains applied to the animal muscle grown *in vitro* it has been concluded that muscle fibers develop evidence of maturity but do not differentiate into the two histochemical types found *in vivo*, probably because they lack motor innervation which seems to be responsible for determination of histochemical types.

Direct physiological-histochemical correlation of individual motor units was studied and parameters of different unit types delineated (in collaboration with LNC, IR) because selective abnormality of the different types of motor units is important in various human neuromuscular diseases. In the cat gastrocnemius, 3 distinct physiologic types were identified and found to correlate completely with histochemistry of the units' muscle fibers. All muscle fibers of a given unit were found to be the same histochemically and they are widely scattered in a muscle, not grouped together.

A new nomenclature for human muscle fiber histochemical types is being developed, since it has become apparant that there are at least 2 subtypes of the two basic histochemical types. The electronmicroscopic details of different muscle fiber types in guinea pig gastrocnemius have been described. The ultrastructure of normal capillaries in human skeletal muscle has been reported.

A system was developed for applying radioisotopic biochemical assays to small samples of biopsied human muscle, utilizing needle or open biopsies and a microslice system. In histochemically fiber-type pure muscle of guinea pigs, red muscle oxidized β-OH-butyrate, succinate, palmitate, and pyruvate more rapidly than white muscle, the differences being most striking for β-OH-butyrate (19 fold) and pyruvate (9 fold). Mitochondrial fractions isolated from guinea pig red muscle showed higher oxidation rates of only β-OH-butyrate and pyruvate, not succinate or palmitate. Much higher activity of the enzyme D(-)β-OH-butyrate dehydrogenase in red vs. white muscle mitochondria was paralleled by similar histochemical findings. Using these techniques, we found in slices of human muscle biopsies that muscle from more than half the patients with spinocerebellar degeneration and 1/3 of cases with idiopathic or familial neuropathy oxidized pyruvate at a rate >5 SE below the control mean. Muscle from 4 patients with myopathies characterized by ragged-red muscle fibers oxidized β-OH-butyrate >3 SE below the control mean.

In mitochondria from muscle of selected patients, absolute respiratory rates and degrees of oxidative-phosphorylation coupling under a variety of conditions were determined (with NIAID).

It was reported that presence of α-keto acids during homogenization of chick embryo liver largely prevented the decrease in tyrosine aminotransferase activity which otherwise occurred, perhaps because they stablize the enzyme by binding to sites different from the catalytic binding site.

Peripheral Neuropathy: Our excellent results with high-single dose alternate-day prednisone treatment in the initial patient with idiopathic recurrent neuropathy improvement maintained now for 41 months, has been extended to several more patients also with improvement, ones with non-remitting as well as ones with remitting courses of disease.

A young lady with the syndrome of continuous muscle fiber activity has been successfully treated with diphenylhydantion for 16 months, even throughout a pregnancy and post-partum period (the first such study during pregnancy).

In a patient with sensori-motor peripheral polyneuropathy, macroglobulinemia and amyloidosis, both high-molecular-weight IgM and low-molecular-weight IgM, were demonstrated and separated by molecular sieve techniques.

Motor Neuron Diseases: In a series of 21 patients with amyotrophic lateral sclerosis (ALS) there were found abnormally very low levels of spinal fluid homovanillic acid (HVA), a product of dopamine metabolism but normal 5-hydroxyindoleacetic acid, a product of serotonin metabolism. Since the probenecid-induced accumulation of HVA was also below normal, a diminished central synthesis of dopamine was suggested. With administration of L-DOPA to 10 ALS patients for 5 months, the CSF HVA rose to very high levels, as did normals, but no clinical improvement was observed. The HVA abnormality is the first metabolic defect observed in ALS patients but its significance to the pathogenesis of the disease remains unknown. Trials of other therapeutic agents in ALS are being formulated.

In ten parents of children with infantile spinal muscular atrophy, a recessively inherited disorder, no subclinical evidence of motor neuron disease was found by muscle biopsy histochemistry or electromyography.

Of 18 histochemical reactions done on the cat lumbar cord in an attempt to learn metabolic particularities of α-motoneurons in the anterior horn of the spinal cord (the ones preferentially involved in ALS), only two distinguished them. α-motoneurons of both fast-twitch and slow-twitch motor units were rich in phosphorylase and poor in succinate dehydrogenase, in contrast to the opposite characteristics of interneurons, γ-motoneurons, and Renshaw cells. Thus one theoretical possibility is that ALS might be related to an abnormality of glycogen metabolism.

A new pathogenesis of some neuromuscular disorders has been hypothesized -- specifically that some erstwhile "myopathic" disorders are actually caused by a lower motor neuron abnormality. It is proposed that in spite of a "myopathic" (which we consider often to be a misnomer) EMG, certain disorders are not myopathic but rather neurogenic. (A new approach to EMG studies is described in the "Methodology" section.) As part of this formulation, details have been proposed regarding how a motor neuron might be defective in a variety of qualitative or quantitative ways with a variety of time courses toward death. Each of these could give a different clinical disorder. Diseases newly considered to be probably neurogenic are cachectic and disuse atrophy, corticosteroid atrophy ("myopathy"), myasthenia gravis, thyrotoxic weakness ("myopathy") myotonic atrophy (myotonic "dystrophy"), type I fiber

These possible mechanisms are being explored. We have reported that the capillary basement membrane by electronmicroscopy is not abnormal, contrasting with a report of thickening by others.

Periodic Paralysis: Acetazolamide (a carbonic anhydrase inhibitor) has been introduced and reported to be the best prophylactic agent available in hypokalemic periodic paralysis. It has thus far benefited 18 of 20 patients, two for over 5 years. It has allowed some patients to discontinue potassium therapy and still remain virtually attack-free. Sixteen of the 18 patients also had moderate or severe persistent proximal weakness between attacks on conventional therapy - with acetazolamide 13 improved, 5 having returned to normal strength. Balance studies and use of other drugs have failed to provide a satisfactory explanation for the mechanism of acetazolamide benefit in this disease; muscle itself has no carbonic anhydrase. Compensated metabolic acidosis occurred in all patients and possibly played a role in the beneficial effect.

The muscle fiber vacuoles characteristic of hypokalemic periodic paralysis were found to be connected to, and presumably dilations of, the T-system rather than the sarcoplasmic reticulum as reported by earlier workers.

A new technique was developed for presenting a high concentration of horseradish peroxidase to a localized area of skeletal muscle in order to completely fill the tortuous T-tubule network in developing avian skeletal muscle fibers prior to electronmicroscopic study. This will be applied to human abnormalities associated with proliferation of the tubular system, such as hypokalemic periodic paralysis.

Neuromuscular Junction Abnormality - Myasthenia Gravis: We have recently presented a new hypothesis on the pathogenesis of myasthenia gravis (MG), to the effect that this is a disease of the lower motor soma involving an inability of it to nourish adequately its distal axonal endings. This is viewed as initially causing a greater defect of excitation factor (acetylcholine) and later a diminution of "trophic factor" too. The neuronal soma defect is proposed to be caused by an altered immunological situation of the thymus and probably other lymphoid tissue. Certain known "immunologic" alterations in MG are by this hypothesis considered parallel and potentially informative phenomena but not pathogenic of the muscle weakness. (One, the serum anti-muscle factor, was reported to be located only in the immunoglobulin G fraction, and since that activity resided only in Fab fragment and not the Fac fragment of the IgG molecule it seemed to be a true antibody.) In accord with this hypothesis, a new treatment has been introduced, high-single-dose alternate-day prednisone. All 4 patients being treated, one for as long as 10 months, have shown remarkable improvement, one to normal and another to virtually normal states. Their anticholinesterase drugs were stopped prior to prednisone and have not needed to be reinstated. This may well prove to be the treatment of choice for many MG patients. Two juvenile MG patients continue do do well on high-single-dose alternate-day prednisone, one having been treated for 3 1/2 years is nearly normal.

Serial No. NDS(I)-62 MN/OC 915(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Histochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators:	W. King Engel, M.D. John R. Warmolts, M.D. Peter Tsairis, M.D.
Other Investigators:	Lowell H. Baker, M.D. R. E. Burke, M.D. Guy G. Cunningham
Cooperating Units:	Laboratory of Neural Control, NINDS

Man Years

Total:	2.7
Professional:	1.2
Other:	1.5

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle, neurons and peripheral nerves and to see how they are altered in neurologic diseases. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To identify selective or non-selective involvement of one histochemical fiber type in the various neuromuscular diseases.

Methods Employed: Histochemical techniques for a number of reactions are being done on muscle and nerve tissue - they have been outlined in previous reports.

Patient Material: Biopsies were obtained from Medical Neurology Branch patients as well as from consultation patients; patient biopsy material was also submitted by outside physicians and investigators.

Major Findings: Direct physiological-histochemical correlation of individual motor units was studied and parameters of different unit types delineated (in collaboration with LNC, IR), because selective abnormality of the different types of motor units is an important phenomenon in various human neuromuscular diseases. In the cat gastrocnemius, 3 distinct physiologic types were identified and found to correlate completely with histochemistry of the unit's muscle fibers. All muscle fibers of a given unit were found to be the same histochemically and they are widely scattered in a muscle, not grouped together.

A new nomenclature for human muscle fiber histochemical types is being developed, since it has become apparent that there are at least 2 subtypes of the two basic histochemical types.

A teaching unit which permits the simultaneous display of audio-taped and slide material was developed to satisfy the numerous persons coming here to learn our methods of clinical research, especially of histochemical studies.

Numerous interrelated studies between this Project and other Medical Neurology Branch Projects were recorded under those other projects, for example:

a. Histochemistry of motor neurons and peripheral nerves - ALS Project.

b. Histochemistry of human and animal muscle cells and neurons in tissue culture - Microbiology Project.

c. Histochemical-electron-microscopic correlation of tubular aggregates, of selective type II muscle fiber atrophy and of experimental regenerating muscle - Electron-microscopy Project.

d. New technique of "Open-biopsy Electromyography" for direct correlation of electromyographic and histochemical data from the same fibers, in patients with various neuromuscular disorders - Electrophysiology Project.

e. Histochemical-electron-microscopic correlation of changes induced in rat muscle by steroids - Myopathy Project.

Significance to Bio-Medical Research and the Program of the Institute: It has been firmly established over the past eight years that histochemistry of muscle biopsies is the single most important laboratory evaluation of human neuromuscular disease, and that it is also extremely useful in (a) screening for correlating with electron-microscopic studies and (b) evaluating animal models. This approach has provided insight into the possibility that certain diseases heretofore called "myopathies" may actually be caused by a form of neuronal abnormality, perhaps related to a defect of neuronal trophic function. This type of analysis (a) helps establish whether the basic pathogenesis and etiology are related to properties reflecting the metabolic sameness or the differentness of the lower motor neurons or muscle fibers, and (b) directs subsequent investigative studies toward analysing those two groups of properties of muscle and nerve cells.

Having further defined the histochemistry of individual motor units in the cat and correlating those results with various electrophysiological parameters, we are better able to understand the normal functions of different types of motor units and how they are altered in disease.

Proposed Course of Project: Further correlations of the results from light-microscopy with those of electron-microscopy, physiology and biochemistry will be made in human neuromuscular diseases and certain normal animal studies. Other animal models of human neuromuscular disorders will be produced and studied. Additional reactions will be studied with the electron probe.

Honors and Awards: None

Publications:

Tsairis, P., Burke, R. E., Levine, D. N., Zajac, F. E. and Engel, W. K.: Histochemical profiles of three physiologically defined types of motor units in cat gastrocnemius muscle. Neurology 21: 436-437, 1971.

Serial No. NDS(I)-62 MN/OC 917(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
R. A. Pieter Kark, M.D.

Other Investigators: John P. Blass, M.D.
Daniel Steinberg, M.D.
Eugene C. Weinbach, M.D.
Alan Borenstein, M.D.

Cooperating Units: Molecular Disease Branch, NHLI
Physiology and Biochemistry Section, LPD, NIAID

Man Years

Total:	1.3
Professional:	1.1
Other:	0.2

Project Description:

Objectives: (1) To seek and analyse biochemical abnormalities of neuromuscular and other neurologic diseases; (2) to elaborate biochemical differences and similarities between "red" and "white" muscle of animals and between type I (similar to "red") and type II (similar to "white") individual muscle fibers of humans and animals.

Methods Employed: Blood, spinal fluid, muscle biopsy, and cultured fibroblast, samples of patients were studied with a variety of techniques:

a. Metabolism of muscle studied by incubating various C^{14} labeled substrates - glucolytic and Kreb cycle intermediates, fatty acids, and amino acids - with human muscle homogenates and cultured fibroblasts and measuring the amounts of labeled CO_2 produced.

b. Standard isotope techniques for oxidation of succinate, β-OH-butyrate, palmitate, and pyruvate have been adapted to tissue slices of small biopsy specimens of muscle.

c. Standard oxygen electrode techniques for the oxidation by pure mitochondrial fractions isolated from human muscle biopsies of succinate, β-OH-butyrate, palmitate, pyruvate, and NADH.

d. Assays for serum and tissue phytanic acid were as previously recorded (Steinberg *et al*., *Ann. Int. Med*., 66: 365-395, 1967).

e. Lactate, pyruvate, and creatine phosphokinase (CPK) of blood and spinal fluid.

f. Enzymes of glycogen metabolism in muscle - phosphorylase, glycogen, amylo-1,6-glucosidase, phosphoglucomutase, and acid and neutral maltase.

Patient Material: Selected Medical Neurology Branch patients with neurological diseases.

Major Findings: a. Simple methods have been developed to facilitate the study of metabolism in normal and diseased human skeletal muscle samples which contain various proportions of two major muscle fiber types. Oxidation of isotopically labeled substrates was measured in microslices (<10 mg wet weight) and in a mitochondrial fraction prepared from as little as 100 mg (but generally 300 to 600 mg) of muscle. The microslice measurements were expressed per unit noncollagen protein. The methods were developed using histochemically pure red (type I fibers, soleus) and white (type II fibers, superficial lateral portion of quadriceps) muscle of guinea pigs. Red muscle microslices oxidized all substrates more rapidly than did white, but the differences were greater for 3-^{14}C-D, L-beta-hydroxybutyrate (nineteenfold) and 2-^{14}C-pyruvate (ninefold) than for 1,4-^{14}C-succinate or 1-^{14}C-palmitate (less than threefold). Moreover, the mitochondrial fraction from red muscle oxidized palmitate and succinate at the same rate as did that from white muscle (per microgram phospholipid phosphorus), but the red muscle fraction oxidized beta-hydroxybutyrate 6 times and pyruvate 2 times as rapidly as did the white. Histochemical results paralleled the biochemical data. These differences suggest that succinate and palmitate oxidation rates possibly reflect the quantity of mitochondria in intact red and white muscle, while the greater differences in beta-hydroxybutyrate and pyruvate oxidation may be indicating qualitative differences of mitochondria in the two types of muscle. Such differences also suggest that differences in motor nerve, known to influence muscle type, may influence the qualitative properties of muscle mitochondria. These techniques have proved applicable to biopsy samples of "normal" human muscle. Microslices of muscle obtained from both open surgical and percutaneous needle biopsies oxidized all 4 substrates reproducibly. Human muscle mitochondrial fractions gave linear oxidation-versus-dilution curves. The proportion of type I:type II muscle fiber volume in each sample was estimated histochemically and compared to the 4 oxidative enzyme activities measured biochemically.

b. In slices of human muscle biopsies we found that muscle from more than half the patients with spinocerebellar degeneration and 1/3 of cases with idiopathic or familial neuropathy oxidized pyruvate at a rate >5 SE below the control mean. Muscle from 4 patients with myopathies characterized by ragged-red muscle fibers oxidized β-OH-butyrate >3 SE below the control mean.

c. In mitochondria from muscle of selected patients, absolute respiratory rates and degrees of oxidative-phosphorylation coupling under a variety of conditions were determined (with NIAID).

d. It was reported that presence of α-keto acids, during homogenization of chick embryo liver, largely prevented the decrease in tyrosine aminotransferase activity which otherwise occurred, perhaps because they stablize the enzyme by binding to sites different from the catalytic binding site.

e. In a family showing hereditary myoclonus epilepsy, a concomitant myopathy with severe morphologic and biochemical (defective NADH oxidation) abnormalities of mitochondria was found. Assuming the same metabolic defect may exist in brain causing the epilepsy, skeletal muscle provides much more accessible tissue for its elucidation.

f. A new syndrome of epilepsy with acanthocytosis, neuromuscular disease, and normal β-lipoprotein has been delineated in a third family.

g. One patient with intermittent cerebellar ataxia, who was found to have pyruvate decarboxylase deficiency, had excessive lipid droplets in his skeletal muscle fibers, as did his clinically normal father. Biochemical abnormalities in other types of spinocerebellar ataxia are noted above.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is important to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity is often neglected but is vital to planning future investigative and therapeutic procedures. Having comparative studies of biochemical differences between type I and type II skeletal muscle fibers will help provide a basis for understanding the pathogenesis of the many neuromuscular disorders preferentially affecting one of these fiber types, as detailed in the Histochemistry Project. With the development of this technique applicable to microslices of human muscle, one can now seek abnormalities of oxidative pathways in human muscle diseases.

Treatment to specifically eliminate from the diet substances improperly handled by patients with concomitant metabolic and neurologic disease, if it continues to be successful, will place hyperphytanicacidemia (Refsum's disease) alongside other metabolic neurologic disorders, e.g., phenylketonuria, galactosemia, and porphyria, which are treated using the same principle.

The spinocerebellar ataxias, usually hereditary, have long been resistant to scientific analysis, but a beginning is now being made in their biochemical analysis.

Proposed Course of Project: The above techniques will continue to be perfected and applied to muscle from a variety of human neuromuscular and central nervous system disorders to seek further details of biochemical defects therein, as well as the biochemical differences and similarities of human type I and type II fibers. It would be facilitated if a technician would become available, as the investigators themselves now must do all the technical work. Treatment of hyperphytanicacidemia will be continued.

Honors and Awards: None

Publications:

Blass, J. P., Kark, R. A. P., Engel, W. K.: Clinical studies of a patient with pyruvate decarboxylase deficiency. Arch. Neurol. In press.

Kark, R. A. P., Engel, W. K., Blass, J. P., Steinberg, D., and Walsh, G. O.: Heredopathia atactica polyneuritiformis (Refsum's disease): A second trial of dietary therapy in two patients. Birth Defects: Orig. 7, No. 1: 53-55, 1971.

Kark, R. A. P., Blass, J. P., and Engel, W. K.: New method for seeking defects in oxidative metabolism. Clin. Res. 19: 141, 1971.

Kark, R. A. P., Blass, J. P., Avigan, J., and Engel, W. K.: The oxidation of β-hydroxybutyric acid by small quantities of type-red and white skeletal muscle. J. Biol. Chem. In press.

DeVivo, D. C. and Peterkofsky, B.: The effect of α-ketoglutarate and other α-keto acids on the recovery of tyrosine aminotransferase activity from chick embryo liver. J. Biol. Chem. 245: 2737-2746, 1970.

Serial No. NDS(I)-63 MN/OC 1034(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigators:	W. King Engel, M.D. Jerry R. Mendell, M.D. John N. Whitaker, M.D. John R. Warmolts, M.D. Nicholas A. Vick, M.D. R. A. Pieter Kark, M.D.
Other Investigators:	Darryl C. DeVivo, M.D. William H. Olson, M.D. Gregory O. Walsh, M.D. Robert I. Roelofs, M.D. Elizabeth Derrer Herbert Y. Meltzer, M.D. John P. Blass, M.D.
Cooperating Units:	Department of Psychiatry, University of Chicago, Chicago, Illinois Molecular Disease Branch, NHLI

Man Years:

Total:	2.1
Professional:	1.6
Other:	0.5

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical, and ultrastructural abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those techniques. To treat these disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological projects of this Branch. Therapeutic trials were conducted with very careful clinical and laboratory examinations to document change of disease state. For comparison with Duchenne muscular dystrophy, structural and non-structural vascular lesions of muscle were produced in animals.

Patient Material: Medical Neurology Branch patients and outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings: In acute psychoses, mostly schizophrenic, a subclinical myopathic degeneration of muscle fibers, found with histochemical techniques, was reported in 22 of 52 patients. It is approximately correlated with peak elevations of the muscle-isozyme of creatine phosphokinase in serum of the male but not female patients. This evidence of an extracerebral component suggests the psychosis may be part of a more generalized metabolic disorder (with Dept. of Psychiatry, U. of Chicago).

A new hypothesis, based on muscle histochemical studies, was reported concerning the pathogenesis of progressive pseudohypertrophic muscular dystrophy of Duchenne. Because the earliest histochemical change is a grouped pattern of degenerating and regenerating muscle fibers, it was proposed that the disease is caused by an abnormality of small arterial blood vessels on either a structural or functional basis. That principle was established and the hypothesis shown to be possible by the production of identical muscle lesions early after embolization of rabbit arteries (structural arterial occlusion) with dextran particles; and repeated embolization over 3 months reproduced the more chronic changes of Duchenne dystrophy. It was pointed out that the subtle, histochemically defined abnormalities in clinically normal carriers of Duchenne dystrophy, namely 1-3 fibers together undergoing necrosis and/or regeneration, are also in accord with this hypothesis and they too were reproduced in the minimally affected regions following embolization. We have now produced the same early and late Duchenne-like muscle lesions with non-structural, presumably functional, lesions of intramuscular blood vessels by combining a subthreshold vascular factor (aortic ligation) and a subthreshold dose of a vasoactive amine (either serotonin or norephinepherin), thereby establishing the possibility of a functional malfunction of blood vessels being the basis of Duchenne dystrophy. Defects of blood vessels and/or vasoactive amines and other substances are now being sought in the patients.

For several of the erstwhile "myopathic" disorders, both long-known ones, such as myotonic atrophy (myotonic dystrophy), and recently found ones, such as type I hypotrophy with central nuclei, a new hypothesis concerning their possibly being caused by a lower motor neuron abnormality has been proposed (see Lower Motor Neuron Disorders).

In progressive external ophthalmoplegia it was found that "ragged red fibers" are present in clinically normal limb muscle (as well as weak muscle) and are characteristic of the syndrome. Such fibers have excessive and morphologically bizarre mitochondria and excessive lipid droplets (see our Electron-Microscopy Project).

A new approach to the over-all classification of neuromuscular diseases was reported.

Muscle biopsies from patients with muscle phosphorylase deficiency (McArdle's disease) have been successfully grown in tissue culture. Their ability to make phosphorylase enzyme is being studied.

Myopathy with emphasis on mitochondrial abnormalities was identified in two siblings with childhood onset myoclonus epilepsy and persistent lactic-acidosis. The mitochondria were bizzare, with whorled cristae and crystalline inclusions.

In the polymyositis/dermatomyositis disease complex, when beginning over the age of 40, especially in males, there is commonly an associated, and perhaps causative, small distant cancer. The childhood form is not associated with cancer, but both age groups might have similar pathogenic mechanisms. The method of treatment we have introduced to this disease, high-single-dose alternate-day glucocorticoid (prednisone), has now proven in our hands to be the best available both for children and adults. It is easier to manage, has greater therapeutic benefit, and fewer side-effects. Our experience extends over 3 1/2 years and includes 20 carefully studied patients. It is effective in patients with or without cancer.

We have proposed a new hypothesis on the mechanism of muscle damage in the polymyositis/dermatomyositis (PM/DM) complex on the basis of our finding deposits of immunoglobulin G, immunoglobulin M, and β_{1C} globulin (C3), alone or in combination, in skeletal muscle blood vessels of 15 of 36 cases of PM/DM, especially in childhood dermatomyositis. We suggest that one of the mechanisms leading to muscle damage in inflammatory myopathy may be vascular abnormality mediated through deposits of immunoglobulin and C3, possibly in the form of immune complexes. This supports our earlier hypothesis that PM/DM is another form of blood-vessel abnormality of muscle. The immunological abnormality could in turn be caused by a viral infection and/or an associated cancer (see our Immunology Project).

Viral isolation studies involving short and long-term cultures of muscle from polymyositis and dermatomyositis patients have so far failed to reveal a transmissible agent (with NCI and PRB, NINDS).

The ultrastructural details of muscle fiber degeneration in polymyositis have been described. Electron-microscopic analysis of capillaries in polymyositis as compared with normal human muscle in the laboratory failed to confirm thickening of the basement membrane reported by others and indicated that it is actually within normal range. Normal human capillaries resembled those of animals; the position of the pericyte was confirmed, indicating a possible major role in blood flow regulation to muscle.

The human myopathies associated with systemic diseases have been reviewed and reported.

The changes induced by corticosteroids in rat skeletal muscle have been delineated electron-microscopically and histochemically.

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electron-microscopy biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represent a step toward the overall understanding of this group of disorders. It is only when the detailed differences of the various forms of myopathy are analysed that the pathogenesis, etiology, and treatment can be understood. In fact, we are now proposing that some erstwhile "myopathies" (such as "myotonic dystrophy") are the result of abnormal neural influence on muscle. Identification of characteristic histochemical and biochemical defects (such as the inability to utilize long-chain fatty acids and phosphorylase deficiency) in the myopathies provides leads to further explore the pathogenesi and possible treatment. The beneficial response obtained in the therapy of polymyositis demonstrates an additional regimen available in the management of such disorders.

The new histologically based hypothesis of ischemia as the cause of Duchenne muscular dystrophy raises for consideration the possible use of drugs to increase the blood flow through perfusion (not shunt) vessels of muscle.

The finding of subclinical myopathy in acute psychoses, mainly of the schizophrenic type, when confirmed as a general phenomenon, provides the basis for considering that both the muscle disease and brain disease (psychosis) are manifestations of a more generalized abnormality; if so, that abnormality may be more easily identified by working with the myopathic aspect.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Honors and Awards: None

Publications:

Vick, N. A.: Skeletal muscle capillary basement membranes in humans. Acta Neuropath. 17: 1-5, 1971.

Engel, W. K.: Duchenne muscular dystrophy: A histologically-based ischemia hypothesis and comparison with experimental ischemia myopathy. Proc. 59th Ann. Mt. Int. Acad. Path. Williams & Wilkins Co., Baltimore, Md. In press.

Walsh, G., DeVivo, D., and Olson, W.: Histochemical and ultrastructural changes in rat muscle. Occurrence following adrenal corticotrophic hormone glucocorticoids and starvation. Arch. Neurol. 24: 83-93, 1971.

Whitaker, J. N. and Engel, W. K.: Vascular deposits of immunoglobulin and complement in inflammatory myopathy. Trans. Amer. Neurol. Ass. 1971. In press.

Engel, W. K.: "Ragged-red fibers" in ophthalmoplegia syndromes and their differential diagnosis. II Intern. Cong. on Muscle Diseases (Nov. 22-26, 1971). In press.

Roelofs, R. I. and Engel, W. K.: Myopathies associated with systemic disease. Postgrad. Med. In press.

Mendell, J. R., Engel, W. K. and Derrer, E. C.: Reproduction of characteristic muscle lesions of Duchenne dystrophy by 5-hydroxytryptamine and norepinephrine in aorta-ligated rats. J. Neuropath. & Exp. Neurol. In press.

Mendell, J. R., Engel, W. K. and Derrer, E. C.: Ischemic myopathy reproducing the histological pattern of Duchenne muscular dystrophy. Neurology 21: 451, 1971.

Mendell, J. R., Engel, W. K. and Derrer, E. C.: Duchenne muscular dystrophy: Functional ischemia reproduces its characteristic lesions. Science 172: 1143-1145, 1971.

Meltzer, H. Y. and Engel, W. K.: Histochemical abnormalities of skeletal muscle in acutely psychotic patients. Arch. Gen. Psychiat. 23: 492-502, 1970.

Engel, W. K.: Classification of neuromuscular disorders. Birth Defects: Orig. 7, No. 2: 18-37, 1971.

Vick, N. A.: The fine structure of polymyositis, with consideration of capillaries and subcellular organelles. Neurology 20: 1062-1068, 1970.

Serial No. NDS(I)-63 MN/OC 1037(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Microbiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Robert I. Roelofs, M.D.

Other Investigators: Jerry R. Mendell, M.D.
Priscilla B. Chauvin

Cooperating Units: None

Man Years

Total:	1.6
Professional:	1.4
Other:	0.2

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electron-microscopic details of animal and human skeletal muscle and motor neurons grown in tissue culture. To compare the growth and histochemical characteristics of "red" vs. "white" muscle in culture. To study interactions between lower motor neurons and muscle fibers in culture. The tissue is from either normal chick, mouse or rat embryo or is normal or abnormal biopsied human tissue, especially muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human cells. Applied to the cultured cells are other techniques being used in the Branch, e.g., histochemistry, electron-microscopy, and immunology.

Patient Material: A portion of the muscle biopsy from certain patients with neuromuscular disease is taken for culture purposes. Needle biopsies of human muscle can also be used.

Major Findings: A large number of experiments were performed in order to obtain optimal cultures for histochemical type-differentiation studies. Refinements in glassware preparation and media composition as well as explanting techniques, have allowed us to now obtain cultures in which numerous mature, striated muscle fibers are found. They are growing for at least 5-8 mm in length and are distinctive from the scanty fibroblastic background.

Reproducible cultures of chick embryo skeletal muscle and spinal cord are being obtained. The histochemical techniques for phosphorylase, myofibrillar ATPase, esterase, glycogen, and several oxidative enzymes have been applied successfully to the cultures. Muscle fibers do develop evidence of maturity in culture without motor nerve supply. Since distinct fiber types by histochemical parameters do not develop in culture, it is concluded that the primary differentiation of muscle fiber types is motor neuron-induced.

Although tissue culture techniques have been used extensively in studying the development of muscle from animal embryos, human skeletal muscle is difficult to culture. In the system we have developed, we achieve reproducible growth for 75% of explants of muscle biopsies of children and adults. The morphologic and histochemical (myofibrillar ATPase and DPNH tetrazolium reductase) development of normal and pathologic human muscle (neuropathies, inflammatory myopathy, central core disease and myophosphorylase deficiency) is similar to that of chick embryo muscle, but is delayed, slower, and has a propensity to form fibers away from the explant.

Preliminary electron-microscopic studies of cultured human muscle to describe the subcellular changes with maturation have been begun.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical properties of normal muscle and its growth characteristics in vitro, the studies of pathologic human muscle in vitro will be used to seek disturbed growth patterns and, more particularly, to determine ways of improving deficient growth. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient with muscle disease to promote growth of abnormal muscle or to retard its degeneration. As a foundation for understanding phenomena in human muscle, chick embryo material has been studied. Study of motor neurons grown in vitro is part of a multilateral approach to diseases which preferentially involve human motor neurons, such as amyotrophic lateral sclerosis.

Proposed Course of Project: Further studies of normal and pathologic human muscle in vitro will be done. Definitive studies, starting with histochemistry, will be applied to the cultured motor neurons. Electron-microscopy of the cultured muscle and nerve cells is in progress.

Honors and Awards: None

Publications:

Roelofs, R. I., Engel, W. K., and Chauvin, P. B.: Growth characteristics of normal and pathologic human muscle in tissue culture. J. Neuropath. Exp. Neurol. In press.

Serial No. NDS(I)-63 MN/OC 1039 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases, Including Peripheral Neuropathies and Spinocerebellar Ataxias

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators:
Justino F. Campa, M.D.
Robert J. Schwartzman, M.D.
Jerry R. Mendell, M.D.
Thomas N. Chase, M.D.
Darryl C. DeVivo, M.D.
R. A. Pieter Kark, M.D.
John P. Blass, M.D.

Cooperating Units:
Molecular Disease Branch, NHLI
Laboratory of Clinical Science, NIMH

Man Years

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: The techniques of histochemistry, biochemistry, electron-microscopy, immunology tissue culture and electromyography were as detailed in other project reports. Additional methods were as follows. Double-blind therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension. Analysis of motor areas of brain and spinal cord by histochemistry and electron-microscopy. Biochemical assays of several parameters of lipid metabolism (with NHLI).

Patient Material: Medical Neurology Branch patients.

Major Findings:

MOTOR NEURON DISORDERS

In a series of 21 patients with amyotrophic lateral sclerosis (ALS) there were found abnormally very low levels of spinal fluid homovanillic acid (HVA), a product of dopamine metabolism, but normal 5-hydroxyindoleacetic acid, a product of serotonin metabolism. Since the probenecid-induced accumulation of HVA was also below normal, a diminished central synthesis of dopamine was suggested. With administration of L-DOPA to 10 ALS patients for 5 months, the CSF HVA rose to very high levels, as did normals, but no clinical improvement was observed. The HVA abnormality is the first metabolic defect observed in ALS patients but its significance to the pathogenesis of the disease remains unknown. Trials of other therapeutic agents in ALS are being formulated.

In ten parents of children with infantile spinal muscular atrophy, a recessively inherited disorder, no subclinical evidence of motor neuron disease was found by muscle biopsy histochemistry or electromyography.

Of 18 histochemical reactions done on the cat lumbar cord in an attempt to learn metabolic particularities of α-motoneurons in the anterior horn of the spinal cord (the ones preferentially involved in ALS), only two distinguished them. α-motoneurons of both fast-twitch and slow-twitch motor units were rich in phosphorylase and poor in succinate dehydrogenase, in contrast to the opposite characteristics of γ-motoneurons, Renshaw cells, and other interneurons. Thus one theoretical possibility is that ALS might be related to an abnormality of glycogen metabolism.

A new pathogenesis of some neuromuscular disorders has been hypothesized -- specifically that some erstwhile "myopathic" disorders are actually caused by a lower motor neuron abnormality. It is proposed that in spite of a "myopathic" (which we consider often to be a misnomer) EMG, certain disorders are not myopathic but rather neurogenic. (A new approach to EMG studies is described in the "Methodology" section.) As part of this formulation, details have been proposed regarding how a motor neuron might be defective in a variety of qualitative or quantitative ways with a variety of time courses toward death. Each of these could give a different clinical disorder. Diseases newly considered to be probably neurogenic are cachectic and disuse atrophy, corticosteroid atrophy ("myopathy"), myasthenia gravis, thyrotoxic weakness ("myopathy") myotonic atrophy (myotonic "dystrophy"), type I fiber hypotrophy with central nuclei and central core disease; ones considered possibly neurogenic (perhaps only in a small part) are benign congenital hypotonia, the facilitating myasthenic syndrome, some cases of facioscapulohumeral disease, and late-onset and congenital rod disease. From such proposals a new classification of neuromuscular disorders has been developed.

A new syndrome of pituitary hypogonadism, Moebius syndrome and subclinical motor neuron involvement of limb muscles was identified.

The generalized muscle cachectic atrophy and weakness which is a common and often the most crippling manifestation of cancer, as well as of hypercorticosterodism (Cushing's syndrome and idiotrophic), and disuse, is selective involvement of histochemical type II muscle fibers. We have postulated this to be a pan-neuropathy of all lower motor neurons to which the type II muscle fibers are more susceptible, not a myopathy as is usually stated. This proposal was supported by our report that the ultrastructure aspects of type II atrophy are qualitatively indistinguishable from typical denervation atrophy, but milder in degree. We will next turn our attention to ways to prevent or treat type II atrophy.

The muscle histochemistry of 3 distinct type of motor units in the cat gastrocnemius have been correlated with their various physiologic properties (with LNC, see our Histochemistry Project).

In a study of cortical representation of sensation in the monkey, it was concluded that neither SI nor SII is of major importance for fine discriminations of tactile, vibratory, proprioceptive, and two-point sensation, contrary to existing neurophysiological theories (with NIMH).

PERIPHERAL NEUROPATHIES

Our excellent results with high-single dose alternate-day prednisone treatment in the initial patient with idiopathic recurrent neuropathy, improvement maintained now for 41 months, has been extended to several more patients also with improvement, ones with nonremitting as well as ones with remitting courses of disease.

A young lady with the syndrome of continuous muscle fiber activity has been successfully treated with diphenylhydantion for 16 months, even throughout a pregnancy and post-partum period (the first such study during pregnancy).

In a patient with sensori-motor peripheral polyneuropathy, macroglobulinemia and amyloidosis, both high-molecular-weight IgM and low-molecular-weight IgM were demonstrated and separated by molecular sieve techniques.

In Refsum's disease (hyperphytanicacidemia), the apparently beneficial effect of dietary regulation (with NHLI) to circumvent the biochemical defect are described in our Biochemistry Project.

SPINOCEREBELLAR ATAXIAS

One patient with intermittent cerebellar ataxia, who was found to have pyruvate decarboxylase deficiency, had excessive lipid droplets in his skeletal muscle fibers, as did his clinically normal father (see our Biochemical Project). Biochemical abnormalities in other types of spinocerebellar ataxia are noted in our Biochemical Project. The various manifestations of the immunologic deficiency state and other metabolic abnormalities in ataxia-telangiectasia, found by us over the past 3 years, have been detailed in a chapter (see our Immunology Project).

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of ALS and other diseases of the lower motor neuron. More accurate evaluation of therapeutic trials are facilitated by (a) development of an instrument for quantitative muscle testing in patients and (b) more complete knowledge of variations in the natural course of the forms of motor neuron diseases. The demonstrated role of abnormal dopamine metabolism in the spectrum of neurologic disorders is being widened to include amyotrophic lateral sclerosis. If dietary treatment of hyperphytanicacidemia (Refsum's disease) continues to be beneficial, another form of neurologic/metabolic disease will have been benefited by circumvention of the known metabolic defect. New aspects of the beneficial use of prednisone in chronic neuropathy indicate additional patients should be tried on the drug on a prolonged program, every-other-day, even if they have been severely paralysed months or longer.

If our proposals are correct that some erstwhile myopathic disorders are actually caused by abnormalities of the lower motor neuron, then all searches for the basic abnormality that are focused on the muscle represent explorations in the wrong forest -- we must direct our attention to the motor neuron rather than the muscle.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders. To further explore the interactions of motor neuron and muscle, and disorders thereof. To undertake additional therapeutic trials.

Honors and Awards: None

Publications:

Campa, J. F. and Engel, W. K.: Histochemical and functional correlations in anterior horn neurons of the cat spinal cord. Science 171: 198-199, 1971.

Campa, J. F. and Engel, W. K.: Histochemistry of motor neurons and interneurons in the cat lumbar spinal cord. Neurology 20: 559-568, 1970.

Engel, W. K.: "Motor Neuron Disease" in Shy, G. M., Goldensohn, E. S., and Appel, S. H., The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Myotonia--a different point of view. Calif. Med. 114: 32-37, 1971.

Engel, W. K. and Warmolts, J. R.: New concepts on the possible role of motoneuron abnormalities in neuromuscular disorders not usually considered neurogenic. In Proceedings of IIémes Journees Internationales de Pathologie Neuro-Musculaire, Marseille, Oct. 28-Nov. 1, 1970, Expansion Scientifique Francaise, G. Serratrice, Ed., in press.

Mendell, J. R., Chase, T. N. and Engel, W. K.: A study of central monoamine metabolism and therapeutic trial of L-DOPA in amyotrophic lateral sclerosis. Arch. Neurol. In press.

Mendell, J. R., Chase, T. N. and Engel, W. K.: A study of metabolism of central monoamines and treatment with L-DOPA in amyotrophic lateral sclerosis. Trans. Amer. Neurol. Ass. In press.

Olson, W. H., Bardin, C. W., Walsh, G. O., and Engel, W. K.: Moebius syndrome: Motor neuron involvement and hypogonadism. Neurology 20: 1002-1008, 1970.

Serial No. NDS(I)-65 MN/OC 1189(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Robert C. Griggs, M.D.

Other Investigators: Jerome S. Resnick, M.D.
Robert J. Schwartzman, M.D.

Cooperating Units; University of Rochester School of Medicine, Rochester, N. Y.

Man Years:

Total:	0.8
Professional:	0.7
Other:	0.1

Project Description:

Objectives: To define more clearly and treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemma-T system-sarcoplasmic reticulum-myofibrillar complex (excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding pertinent metabolic pathways and methods of treatment.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron-microscopy, and biochemical assays of tissue were delineated in our other projects. Provocative loading tests and therapeutic trials to raise or lower potassium or sodium are used. Acetazolamide, triamterene, and ammonium chloride were administered as possible prophylactic agents for hypokalemic periodic paralysis. Electrolyte content and balance were evaluated on and off therapy.

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Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbance; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita. (Patients with myasthenia gravis are part of another Medical Neurology Branch Project.)

Major Findings: Acetazolamide (Diamox), introduced by us as a new prophylaxis in hypokalemic periodic paralysis, was reported to continue to be very effective with no significant side effects in 18 of 20 patients, two followed for over five years. Eighteen of the 20 patients with hypokalemic periodic paralysis, both sporadic and familial, have responded with dramatic decrease in attack frequency - 10 who had had almost daily attacks despite large doses of prophylactic potassium have had no attacks on acetazolamide. The study was single-blind, placebo-controlled, with frequent cross-overs. Sixteen of the 18 had had moderate or severe persistent proximal weakness between attacks even on conventional therapy - on acetazolamide 13 improved, 5 of these having returned to normal strength. Some have discontinued their potassium therapy.

The mechanism of acetazolamide benefit in this disorder is unknown. Skeletal muscle is thought not to contain carbonic anhydrase. Studies in these patients indicated acetazolamide did not significantly alter thyroid function. Balance studies showed the drug to cause an initial increase of potassium excretion but no long-term change of total body potassium despite persistent therapeutic effect. Similarly, despite slightly increased initial excretion of sodium, no eventual effect on total exchangeable body sodium was found. Another diuretic, triamterene, which produced a greater natruresis, had no therapeutic effect. All patients have a moderate, compensated metabolic acidosis.

The muscle fiber vacuoles characteristic of hypokalemic periodic paralysis were found to be connected to, and probably dilations of the T-system rather than the sarcoplasmic reticulum as reported by others.

Significance to Bio-Medical Research and the Program of the Institute: Our introduction of acetazolamide in the treatment of hypokalemic periodic paralysis has provided the most effective prophylaxis available in this disorder. It remains reliably effective and safe over a long period of time (more than 5 years to date in some patients). It benefits about 90% of the patients tested. This new form of prophylaxis provides new avenues for investigation of the basic pathophysiology in this disease.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even more effective therapeutic agents.

Serial No. NDS(I)-65 MN/OC 1189(c)

Honors and Awards: None

Publications:

Griggs, R. C., Engel, W. K., and Resnick, J. S.: Acetazolamide treatment of hypokalemic periodic paralysis. Ann. Intern. Med. 73: 39-48, 1970.

Serial No. NDS(I)-65 MN/OC 1190(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Myasthenia Gravis

Previous Serial Number: Same

Principal Investigators: John R. Warmolts, M.D.
W. King Engel, M.D.

Other Investigators: Dale E. McFarlin, M.D.
John S. Johnson, M.D.

Cooperating Units: Laboratory of Clinical Investigation, NIAID

Man Years

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: To apply histochemical, pharmacologic, electrophysiologic and immunologic techniques to investigate the pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: Clinical studies, including prostigmine and curare challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases. Competence of neuromuscular transmission was studied neurophysiologically in patients with myasthenia gravis and other neuromuscular disorders. A new treatment program consisting of high-single-dose (100mg for the average adult) alternate-day prednisone was introduced, with adjuncts of supplemental potassium, antacids, and low sodium, low carbohydrate and high protein diet.

Sera from patients with myasthenia gravis were studied by a variety of immunologic techniques, including agar diffusion, immunoelectrophoresis, latex agglutination, gel filtration, ion exchange chromatography, and protolytic digestion of proteins. Specifically, the antimuscle factor (AMF) in the serum of 5 patients was studied to determine which part of the AMF molecule reacted with skeletal muscle. $F(ab)_2$ fragments were prepared by isolation of IgG containing AMF and subsequent digestion with pepsin. Lymphocytes and thymocytes were grown in short-term tissue culture and their responsiveness to common cellular stimulants was measured by incorporation of tritiated thymidine in DNA. Specimens of muscle, and in some cases thymus and thyroid, were studied using immunofluorescent, histochemical and routine histological techniques. The abnormalities were evaluated before and after thymectomy.

Patient Material: Patients participated in the therapeutic trials. Sera, muscle, thymus and other tissue were obtained from Medical Neurology Branch patients.

A complete hypothesis utilizing data from many types of investigative studies has been proposed, to the effect that myasthenia gravis is a disorder of the lower motoneuron soma, which fails to provide a qualitatively and/or quantitatively adequate axoplasmic flow to nourish the axon tips. This motoneuron defect is proposed as being caused by an abnormality of thymus and related tissues. Certain known "immunologic" alterations in MG are by this hypothesis considered parallel and potentially informative phenomena but not pathogenic of the muscle weakness. The motoneuron defect is viewed as initially causing a greater defect of excitation factor (acetylcholine) and later a diminution of "trophic factor" too.

A patient with juvenile myasthenia gravis has now been maintained at about 95% improvement for 4 years on corticosteroids, the last 2 1/2 years on a program new for this disease, high-single-dose alternate-day prednisone. A second juvenile myasthenic patient has also shown beneficial response for 16 months. This is in contrast to the short course of ACTH customarily given for adult myasthenia gravis.

A new and very successful treatment has been introduced for adult myasthenia gravis, namely the high-single-dose alternate-day prednisone. In our first four patients thus far treated, this has proved to be much more effective therapy than anticholinesterase drugs (which we discontinue when prednisone is begun). One patient has been treated for a year and is now virtually normal. Side effects have been virtually absent.

The electrophysiologic studies of neuromuscular transmission before and during alternate-day prednisone treatment are detailed in our Clinical Neurophysiology Project.

The antimuscle factor(AMF) was shown to reside in the Fab fragment but not the Fac fragment of the IgG molecule. This finding supports the concept that AMF is an antibody and that an autoimmune reaction is present in the 30% of myasthenia gravis patients who have this serologic abnormality in their serum.

Short-term treatment of one far advanced case of myasthenia gravis with ACTH produced striking improvement. During this treatment no change in the absolute lymphocyte count or titer of AMF was seen.

Thymocytes from one patient with myasthenia gravis responded to phytohemagglutinin and poke weed mitogen in a fashion similar to that of peripheral blood lymphocytes, unlike normal thymocytes.

Significance to Bio-Medical Research and the Program of the Institute: The response of myasthenic patients to alternate-day prednisone indicates that much of the abnormality is not irreversible and supports the idea that improvement can result even in the most severely affected and advanced stages of this disease. The demonstrated benefit of alternate-day prednisone in juvenile myasthenia gravis led us to apply this new program to adult myasthenia gravis patients. It has resulted in a remarkable new method of treatment, using a drug that has been and is still listed in some sources as "contraindicated" in myasthenia gravis. We consider alternate-day prednisone the new treatment of choice in myasthenia gravis and believe it offers the possibility of suppressing the disease effectively for a sufficiently long period for it to disappear completely. If this will occur, the disease would be cured in such a patient.

The demonstration that the antimuscle factor in patients with myasthenia gravis does not differ immunochemically from the antimuscle factor in patients with thymoma and no myasthenia gravis supports our suspicion that this agent per se is not responsible for the neuromuscular block in this disease. The demonstration that antimuscle factor is due to the Fab portion of the IgG molecule supports the concept that this serological reactivity is an antibody and thus develops as part of an immunological process.

Finding in the thymus of a patient with myasthenia gravis cells which respond to stimulants supports the notion that reactive cells are present in thymus of patients with this disease. This is consistent with the concept of a "thymitis" and is in contrast to normal thymus in which the thymocytes are non-responsive.

Proposed Course of Project: The various clinical and basic mechanistic aspects of our new alternate-day prednisone program will be investigated more extensively. Neurophysiologic studies will be done more extensively.

Honors and Awards: None

Publications:

Engel, W. K. and Warmolts, J. R.: Myasthenia gravis: A new hypothesis of the pathogenesis and a new form of treatment. Ann. N. Y. Acad. Sci. In press.

McFarlin, D. E. and Johnson, J. S.: Studies of antimuscle factor in myasthenia gravis. II. Reactions of fragments produced by enzymatic digestion. J. Immunol. 106: 292-294, 1971.

Warmolts, J. R., Engel, W. K., and Whitaker, J. N.: Remarkable benefit from alternate-day prednisone treatment in myasthenia gravis. Lancet II: 1198-1199, 1970.

Warmolts, J. R. and Engel, W. K.: Benefit from alternate-day prednisone in myasthenia gravis. Neurology 21: 412, 1971.

Serial No. NDS(I)-65 MN/OC 1191(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunological Abnormalities of Neurologic Diseases

Previous Serial Number: Same

Principal Investigators: John N. Whitaker, M.D.
W. King Engel, M.D.

Other Investigators: Darell D. Bigner, M.D.
Dale E. McFarlin, M.D.
John S. Johnson, M.D.
Thomas A. Waldmann, M.D.
Warren Strober, M.D.
John L. Sever, M.D.

Cooperating Units: Metabolism Branch, NCI
Laboratory of Clinical Investigation, NIAID
Perinatal Research Branch, NINDS

Man Years

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the central nervous system and neuromuscular system.

Methods Employed: Standard immunochemical techniques were employed for studying both immunoglobulin and cellular aspects of altered immune states.

Patient Material: Specimens of peripheral blood, bone marrow, muscle, peripheral nerve, thymus and other tissue was obtained from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: The various manifestations of the immunologic deficiency state and other metabolic abnormalities in ataxia-telangiectasia, found by us over the past 3 years, have been detailed in a chapter.

Low-molecular-weight (LMW) IgM was found in a patient with macroglobulinemia and amyloid neuropathy. In contrast to that seen in a patient with recurrent encephalopathy, the LMW-IgM was rather homogenous in electrophoretic mobility and monotypic. Characterization of the protein abnormalities in this patient provide explanation for discrepancy between IgM levels and lack of symptoms related to serum hyperviscosity seen in other patients with macroglobulinemia. From the findings in this patient a hypothesis concerning one mechanism for the remote effects of cancer on the CNS was put forth, namely that it is an antibody activity of serum monotypic HMW and/or LMW-IgM directed against nervous tissue.

In a patient with subacute sclerosing panencephalitis (SSPE) and high titers of measles antibody, treatment with immunocompetent lymphocytes from a normal sibling was given but found unsuccessful (with PRB-NINDS and NCI). In another patient with that disease, two treatment attempts using transfer factor from the patient's twin were not successful (with PRB-NINDS). Immunoglobulins localized in blood vessels from 2 of 3 such patients raise the possibility of a pathogenic role of circulating immune complexes in that disease.

Specimens collected from every Medical Neurology Branch patient and stored in the serum and spinal fluid bank have proven to be valuable in the study of a number of entities of possible viral and/or autoimmune etiology.

On the basis of our finding deposits of immunoglobulin G, immunoglobulin M, and β_{1C} globulin (C3), alone or in combination, in skeletal muscle blood vessels of 15 of 36 cases with the polymyositis/dermatomyositis (PM/DM) complex, especially in childhood dermatomyositis, we have proposed a new hypothesis on the mechanism of muscle damage in the PM/DM complex. We suggest that one of the mechanisms leading to muscle damage in inflammatory myopathy may be vascular abnormality mediated through deposits of immunoglobulin and C3, possibly in the form of immune complexes. This supports our earlier hypothesis that PM/DM is another form of blood-vessel abnormality of muscle. The immunological abnormality could in turn be caused by a viral infection and/or an associated cancer (see our Myopathy Project).

For the immunologic aspects of myasthenia gravis, see our Myasthenia Gravis Project.

Significance to Bio-Medical Research and the Program of the Institute: The new possibility that immune complexes may be related to the pathogenesis of at least some cases of the polymyositis/dermatomyositis complex may lead to new therapeutic approaches.

Elucidation of the defects responsible for abnormalities in ataxia-telangiectasia may lead to learning the mechanism responsible for the nervous system pathology. Whether the underlying immunological defect present in ataxia-telangiectasia predisposes to an infection ultimately responsible for the central nervous system disease remains to be seen. Viral studies on our patients have thus far been negative. All data to date favor a primary inherited metabolic defect in this disease, which in turn causes the immunologic abnormalities. The mechanism in this disease, when elucidated, may have a direct bearing on the pathogenesis of multiple sclerosis and other acquired diseases of the brain.

Subacute sclerosing panencephalitis has come to be considered an effect of the measles virus - a lingering infection with a "slow" form of virus or an altered immune response to the virus, or perhaps both. These concepts led to the therapeutic attempts noted, which were unsuccessful. Yet the treatment and prevention of various chronic neurologic diseases postulated to be related to viruses may well evolve from such theoretically based therapeutic trials.

Low molecular weight IgM may be of considerable importance in infectious processes involving the central nervous systems. Some antigens elicit an antibody response in only one antibody class. When the antibody class is IgM, the resulting antibody would have some difficulty entering the central nervous system, if it were the usual high molecular weight gamma M which has a high molecular weight and high diffusion coefficient. However, the low molecular weight gamma M under study is smaller in size and has a lower diffusion coefficient and thus would be expected to enter the CNS more readily. This finding gives added impetus to the study of a possible pathogenic role low grade infectious organisms in chronic central nervous system "degenerative" disorders.

Proposed Course of Project: The immunological defects in the polymyositis/dermatomyositis complex, in SSPE, and in some neuropathies are to be elucidated more fully with the addition of the latest immunological techniques. Additional studies of immune states in a number of other neurological conditions are planned.

Honors and Awards: None

Publications:

McFarlin, D. E., Strober, W., Barlow, M. H., and Waldmann, T. A.: Immunological abnormalities in ataxia-telangiectasia. Proc. Ass. Res. Nerv. Ment. Dis. In press.

Bigner, D. D. and McFarlin, D. E.: Neurology of immunoglobulin anbormalities. Proc. Ass. Res. Nerv. Ment. Dis. In press.

Bigner, D. D., Olson, W. H., and McFarlin, D. E.: Peripheral polyneuropathy, high and low molecular weight immunoglobulin M, and amyloidoses. Arch. Neurol. 24: 365-373, 1971.

Serial No. NDS(I)-65 MN/OC 1192(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron-Microscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators:
W. King Engel, M.D.
Jerry R. Mendell, M.D.
Donald Bishop

Other Investigators:
Nicholas A. Vick, M.D.
William H. Olson, M.D.
Michel Reznik, M.D.
Mary Ann E. Oberc
Guy G. Cunningham
Alan M. Rapoport, M.D.
Michel Fardeau, M.D.

Cooperating Units:
Armed Forces Institute of Pathology, Washington, D. C.
Service de Microscopie Electronique, Paris, France

Man Years

Total:	3.2
Professional:	2.0
Other:	1.2

Project Description:

Objectives: To study the early subcellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle, such as denervating and metabolic diseases. To study subcellular changes of human neurons and peripheral nerves in certain diseases.

Methods Employed: A portion of biopsied muscle is tied to a glass rod to maintain resting length and immediately fixed in cold buffered glutaraldehyde or osmium tetroxide for eventual araldite embedding. Other fixative combinations and buffers have also been experimented with. Various electron-microscopic (EM) staining procedures are used. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in the Histochemistry Project. Histochemically stained cryostat sections are ready within half an hour after the biopsy and are used as a guide to determine which of the biopsies contain light microscopic features of special interests and thereby serve as a guide for choosing material appropriate for electron-microscopic study. Biopsy specimens of human nerve or brain tissue are fixed and embedded for electron-microscopy and are similarly monitored by rapid enzyme histochemistry of adjacent tissue. Skin samples and cultured fibroblasts from our patients are sometimes studied. In the animal models studied in parallel to the human diseases, tracer substances of peroxidase, ferritin, and lanthanum are sometimes used.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are the major sources of material. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Muscle biopsies and nerve biopsies are the major tissues examined. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease. Other abnormal tissues from neurologic patients are occasionally examined, such as skin and cultured fibroblasts.

Major Findings: Aggregates of material rich in TPNH and DPNH-tetrazolium reductase, devoid of succinate dehydrogenase, and devoid of menadione-mediated α-glycerophosphate dehydrogenase are confined to type II human muscle fibers. This change was first described from this Branch. The ultrastructure has now been correlated with histochemistry in the same patients. The aggregates, termed "tubular aggregates", consist of masses of tubules, often with a single coaxial innertubule. They appear to be massive proliferations of sarcoplasmic reticulum, viz. longitudinal component and/or lateral sac. They demonstrate a new reactivity of sarcoplasmic reticulum and a new opportunity to study histochemically the reticulum away from the mitochondria. The rich DPNH and TPNH-tetrazolium reductases they contain is attributable to "microsomal" DPNH and TPNH cytochrome c reductases. They further have led to the hypothesis that normally these enzymes of the sarcoplasmic reticulum may help supply for SR ATP synthesis to drive the SR calcium pump. Tubular aggregates are found in patients with periodic paralysis and others taking large amounts of drugs.

To support the hypothesis of vascular abnormality in Duchenne muscular dystrophy, certain morphologic abnormalities have been found in intramuscular blood vessels (see our Myopathy Project).

Details of mitochondrial abnormalities - enlargement, amorphous lipid-like inclusions, organized box-car-like inclusions between the inner and outer membrane, and contorted cristae - have been elucidated in six unusual kinds of muscle disease. In some cases these are paralleled by biochemically demonstrated defects of mitochondrial oxidative enzyme activity.

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases has been initiated, in an attempt to confirm one report by another group. To date, myxovirus-like structures have been found in some cases of polymyositis.

Ultrastructural analysis of blood vessels in patients with collagen-vascular-disease forms of polymyositis (including dermatomyositis) so far fails to confirm a report from another laboratory that thickening of the intramuscular capillary basement membrane is characteristic of this disease group. In fact, it has been shown that the width of basement membrane which others have considered to be abnormal is actually within the normal range.

The ultrastructural details of muscle fiber vacuolation and degeneration in polymyositis have been reported.

From a correlative study of regenerating muscle fibers following experimental injury in animals, it was reported that ultrastructural changes are predictable from histochemical findings. In general, mitochondria were much more susceptible than myofibrils, the reverse of what is found in denervation atrophy.

The simpler synaptic structure of the hippocampus makes it more suitable for study of epileptic phenomena than neocortex. A powerful recurrent inhibitory system in the hippocampus is known to limit interictal epileptic discharges temporally and spatially. The synaptic input on neuronal somas and large dendrites of the CA_4 area of the cat hippocampus, a key region, is being studied electron-microscopically to provide a morphological basis for structure-function correlations.

The electron-microscopic appearance of muscle biopsies from patients with type II muscle fiber atrophy is described. The ultrastructural changes are qualitatively indistinguishable from denervation atrophy but are milder in degree. These findings suggest that type II atrophy represents an alteration of the normal neural influence on skeletal muscle fibers, perhaps of a trophic factor.

The electron-microscopic details of different muscle fiber types in guinea pig gastrocnemius have been described.

The ultrastructure of normal capillaries in human skeletal muscle has been reported.

Horseradish peroxidase was used as a cytochemical tracer to outline the T-system of the chicken pectoralis muscle. A new technique was developed whereby a high concentration of horseradish peroxidase was presented to a localized area of skeletal muscle completely filling the T-system and revealing a tortous, complex tubular network different from that of other vertebrates. This same technique can be applied to the study of human muscle disease in which there is a proliferation of the tubular system, e.g., periodic paralysis and denervation atrophy.

Electron-microscopic studies of human skeletal muscle grown in tissue culture are referred to in our Microbiology Project, and of muscle from rats with steroid-induced changes in the Myopathy Project.

"Ragged red fibers", identified histochemically (rich in neutral lipid and mitochondrial oxidative enzymes) in clinically normal skeletal muscle as the most common lesion in patients with slowly progressive external ophthalmoplegia, have been described electron-microscopically - they contain excessive amounts of lipid droplets in the sarcoplasm and groups of abnormal mitochondria within which is amorphorus granular material.

Rous sarcoma virus-induced brain tumors from dogs and hamsters are being looked at to compare their ultrastructure and possible viral particle content with "spontaneously" arising (? viral) brain tumors of humans. To date, no viral particles were found in those animals, but they were seen when the tumors were transferred back to avians via extracts (see our Brain Tumor Project).

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its infancy. Identification of new forms of disease occur rapidly in this stage. However, one must be careful not to name a condition on the basis of careful though limited EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is close correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, as in "tubular aggregates" of sarcoplasmic reticulum and crystalline and other changes of mitochondria. Other ultrastructural changes, e.g., accumulation of lipid in otherwise normal fibers, provide clues as to which metabolic pathways are likely to be the site of a presumed enzyme defect. These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. Electron-microscopy is also indispensible for the detailed morphologic analysis of topics listed under our other projects, e.g., the vascular-etiology hypotheses of Duchenne muscular dystrophy and polymyositis, and the viral-etiology hypothesis of animal and human brain tumors.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic, viral, and tissue culture techniques with electron-microscopy applied to human neurologic diseases and animal models have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Honors and Awards: None

Publications:

Engel, W. K., Bishop, D. W. and Cunningham, G. G.: Tubular aggregates in type II muscle fibers: Ultrastructural and histochemical correlation. J. Ultrastruct. Res. 31: 507-525, 1970.

Fardeau, M.: Etude ultrastructurale des differents types de fibres musculaires dans la musculature squelettique du cobaye. Proc. Conf. Marseille, Oct. 28-Nov. 1, 1970. In press.

Mendell, J. R.: Unusual feature of the T-system of the pectoralis muscle of the chicken. J. Ultrastruct. Res. In press.

Mendell, J. R. and Engel, W. K.: The fine structure of type II muscle fiber atrophy. Neurology 21: 358-365, 1971.

Schulman, J. D., Wong, V., Olson, W. H., and Seegmiller, J. E.: Phagolysosomal site of crystalline deposits in cystinosis as shown by ferritin uptake. Arch. Path. 90: 259-264, 1970.

Reznik, M. and Engel, W. K.: Ultrastructural and histochemical correlation of experimental muscle regeneration. J. Neurol. Sci. 11: 167-185, 1970.

Vick, N. A.: The fine structure of polymyositis, with consideration of capillaries and subcellular organelles. Neurology 20: 1062-1068, 1970.

Vick, N. A.: Skeletal muscle capillary basement membranes in humans. Acta Neuropath. 17: 1-5, 1971.

Serial No. NDS(I)-65 MN/OC 1193(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To apply radioautographic techniques to the normal and abnormal neuron and muscle cell metabolism. A specific topic is the study of alterations in protein metabolism of motor and sensory neuron in experimentally induced neuropathies.

Methods Employed: Radioautography is employed, using the excellent resolution afforded by the low energy β-radiation of tritiated amino acids. Labeled proteins are located in the central and peripheral nervous systems of muscle at intervals following administration of tritiated amino acids.

Patient Material: Patient material has not yet been utilized.

Major Findings: In last year's annual report the pattern of distribution of radioactivity in dorsal and ventral nerve roots after administration of tritiated leucine was described as indicating that in normal cats there was a flow of labeled proteins from site of synthesis in neuronal cell bodies along axonal processes at approximately 1.5 mm/day. This flow was interrupted in cats made neuropathic by acrylamide but remained normal in those with tri-orthocresyl phosphate induced neuropathy.

Preliminary biochemical studies indicated that the labeled substance flowing along axons has a molecular weight greater than 1500 and is rather strongly bound to DEHE-cellulose at pH 7.0.

It was hoped to extend such studies and perform other radioautographic investigations, but lack of personnel and space did not permit it. Hence, this report has been dormant this year.

Significance to Bio-Medical Research and the Program of the Institute: The findings of last year indicated that the apparently selective axonal destruction induced by one toxin (acrylamide) may be due to a failure of synthesis by the neuron cell body of proteins essential for axonal integrity. Consequently certain inherited and metabolic diseases of the human nervous system with similar selective axonal destruction may have the same pathophysiologic defect.

Proposed Course of Project: Hoped for extensions of this study of axonal flow include the separation and characterization, by biochemical methods, of the proteins that are synthesized in the cell bodies of neurons and destined for export to their axonal processes. However, this will necessitate both the return of the position allocations for a co-principal investigator and a technician, as well as space in which to work.

Honors and Awards: None

Publications: None. (See last year's report).

Serial No. NDS(I)-69 MN/OC 1728(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Brain Tumor

Previous Serial Number: Same

Principal Investigators:	Darell D. Bigner, M.D. John P. Kvedar, A.B. W. King Engel, M.D.
Other Investigators:	Nicholas A. Vick, M.D. W. Ray Bryan, Ph.D. Milton W. Brightman, Ph.D. Thomas S. Reese, M.D.
Cooperating Units:	Viral Oncology Branch, NCI Duke University School of Medicine, Department of Neurology, Durham, N.C. Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS

Man Years

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objectives: (1) To develop a brain tumor animal model in which to study the pathogenesis and better methods of diagnosis of brain tumors; (2) to develop the most sensitive animal system in which to attempt recovery of oncogenic viruses from human brain tumors; (3) to improve methods of virus extraction from SR-Rous sarcoma virus (SR-RSV) tumors; and (4) to develop new methods of treating brain tumors.

Methods Employed: (a) Assay of SR-RSV, an RNA tumor-inducing virus, on the chorioallantoic membrane of chicken eggs; (b) preparation of high-potency, bacteria-free suspensions of SR-RSV by a new combination of buffer systems, differential centrifugation, and membrane filtration; (c) induction of brain tumors in young dogs or hamsters by injecting the high-potency suspensions of SR-RSV; (d) measurement of antibody responses in tumor-bearing dogs, demonstration of SR-RSV antigen in the induced brain tumors; (e) recovery of SR-RSV from the tumors in chick embryo fibroblast tissue culture, in chicken, or in Japanese quail; (f) measurement of the effect of various drugs on reducing the amount of SR-RSV necessary from brain tumor induction; (g) morphologic study of the SR-RSV-induced brain tumors and their cells shed into the spinal fluid with histochemical, electron-microscopic, light-microscopic, and immunofluorescent techniques, seeking (1) viral particles and (2) details of the morphologic changes of the neoplastic cells; (h) brain scanning of tumor-bearing animals with various isotopes.

Patient Material: None

Major Findings: An improved method of concentrating and purifying Schmidt-Ruppin Rous avian sarcoma virus (SR-RSV) yielded, 4 weeks after intracerebral inoculation into newborn dogs and hamsters, 96% survivor incidence and 100% brain tumor incidence in survivors, compared to 49% and 86% respectively with previous methods. A quantitative dose-response relationship was demonstrated. The tumors were sarcomas and gliomas (spongioblastomas, astrocytomas, and ependymomas) with varying degrees of anaplasia. Injection of virus near the cerebral surface or in the cerebellum led to sarcomas and deep cerebral periventricular injection to gliomas. No evidence of virus was found in dog brain tumors with electron-microscopy or immunologic methods, but the SR-RSV genome was detected by inoculating whole cell suspension of dog and hamster brain sarcomas and gliomas back into avian hosts, wherein typical Rous sarcomas containing C-type viral particles, group specific viral antigens, and infectious RSV were induced. The ultrastructural details of cell types composing these brain tumors have been described.

Significance to Bio-Medical Research and the Program of the Institute: These induced brain tumors are an especially suitable system in which to study mechanisms that allow malignant transformation of glial and mesodermal brain cells by RNA-C-type viruses without detectable evidence of viral replication, as is likely to be occurring in at least some spontaneous human brain tumors. This system will serve as a guide to isolation of viruses causing human brain tumors. The reproducibility and rapidity of experimental tumor induction, type of blood supply, and histologic similarity of the experimental gliomas to spontaneous human gliomas make these SR-RSV-induced animal brain tumors ideal for morphologic, chemotherapeutic and brain-scanning studies. They are now being used to study (with LNNS, IR) the blood-brain-barrier changes in tumors, combining tracer substances with electron-microscopy.

Perhaps the most important implication is the fact that virally induced brain tumors that have no evidence of viral particles or viral antigens, but which do contain viral genetic material, are available for study, since these same conditions may be operative in spontaneous human brain tumors.

Proposed Course of Project: Ideally the project should be continued in several directions. First, the technology of concentrating SR-RSV should be extended to extraction of spontaneous human brain tumors for inoculation of animals to try to demonstrate the viral etiology of human tumors. In addition, the basic cellular mechanisms that allow malignant transformation of cells with our detectable evidence of viral antigens or mature virus should be studied and applied to the human situation. Also, the ability to induce large brain tumors in large and small animals should be exploited for evaluating chemotherapeutic agents and better diagnostic methods, such as brain scanning.

Honors and Awards: None

Publications:

Bigner, D. D., Vick, N. A., Kvedar, J. P., and Engel, W. K.: Correlated virological and ultrastructural study of Schmidt-Ruppin Rous sarcoma virus-induced dog brain tumors. J. Neuropath. Exp. Neurol. 30: 122-123, 1971.

Brightman, M. W., Reese, T. S., Vick, N. A., and Bigner, D. D.: A mechanism underlying the lack of a blood-brain-barrier to peroxidase in virally induced brain tumors. J. Neuropath. Exp. Neurol. 30: 139-140, 1971.

Bigner, D. D., Vick, N. A., Kvedar, J. P., and Engel, W. K.: Gliomas and intracranial sarcomas induced in dogs and hamsters with Schmidt-Ruppin Rous sarcoma virus. Proc. VI Int. Cong. Neuropath., Masson et Cie, Paris, 568-569, 1970.

Vick, N. A., Bigner, D. D., and Kvedar, J. P.: The fine structure of canine gliomas and intracranial sarcomas induced by the Schmidt-Ruppin strain of the Rous sarcoma virus. J. Neuropath. Exp. Neurol. In press.

Serial No. NDS(I)-70 MN/OC 1792(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrophysiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigator: John R. Warmolts, M.D.

Other Investigators: W. King Engel, M.D.
Dennis L. Martin, M.D.

Cooperating Units: None

Man Years

Total: 0.8
Professional: 0.8
Other: 0.0

Project Description:

Objectives: (1) To develop more precise methods of electromyography (EMG) recording and analysis; (2) to establish better EMG indices of early myopathic or neuropathic involvement; (3) to improve the value of the EMG in distinguishing the neural or myopathic basis of atypical disorders; (4) to investigate mechanisms regulating motor unit recruitment in normal subjects and those with neuromuscular disorders; (5) to correlate the electrical behavior of motor units with their histochemically defined properties; (6) to use clinical electrophysiologic parameters to evaluate course of disease and response to treatment.

Methods Employed: Recordings are made in humans using the Teca-Medelec electrophysiologic system. Permanent data are stored by high speed magnetic tape recording and photographic recordings. Computer frequency analysis programs are run. Correlation studies are directed toward the (a) recruitment threshold, (b) rank order of recruitment, (c) preferential stable discharge frequency, (d) relative rhythmicity, (e) amplitude, (f) duration, and (g) configuration of EMG recorded individual motor unit action potentials in human subjects with a variety of neuropathic and myopathic conditions, and in subjects without definable neuromuscular disease.

A variety of parameters of neuromuscular transmission are repeatedly evaluated in myasthenia gravis patients before and during treatment. Partial curarization of subjects with various fatigue states have been performed with attention directed primarily to the behavior of single motor units.

Patient Material: Patients with a variety of neuromuscular disorders and without definable neuromuscular disease from the Medical Neurology Branch and consultation service.

Major Findings: Four new aspects of clinical electromyography (EMG) have been developed, facilitated by high-speed tape recording: (a) A new descriptive term for the pattern of short duration, small amplitude, excessively abundant potentials for a given amount of voluntary effort, viz., "SSAP", was introduced to replace the generally used term "myopathic", a designation which we consider to be misleading at times. We consider SSAPs just as likely to be due to neuronal disease as to myopathic disease. (b) The new technique of "Open Biopsy Electromyography" was introduced to provide tight correlation of electrophysiology done by EMG recording of the same muscle fibers excised immediately after recording and studied histochemically. (c) Using this technique in patients with biopsies showing predominance of either type I or type II muscle fibers, we have identified the different physiological properties of these two human motor unit types. Type I motor units fire with initial effort and discharge more regularly and more continuously, while type II units fire only irregularly in unsustained bursts and only when more vigorous contraction is exerted. (d) The electromyographic patterns of selective type II and type I muscle fiber atrophy in humans have been identified, and an hypothesis developed that relates the electrical data to the neurogenic origin we propose for these atrophies.

A fixed order of recruitment of motor unit action potentials (MUAPs) from motor units during sustained isometric limb contractions has been reported in both proximal and distal, upper and lower limb muscles that is dependent upon the strength of voluntary contraction. Succeeding MUAPs appearing on graduated isometric contraction demonstrate respectively increasing amplitude and duration. Variations from this occur when action potentials of different motor units are of very near size and threshold. No difference in stable discharge rhythmicity has been correlated with order of recruitment or size among the first several motor unit potentials recruited at any one recording point in muscle. Continuing observations are being made on this topic.

On the basis of correlated electromyographic and histochemical study of certain diseases and in comparison to theoretical possibilities, it is proposed that what is currently termed a "myopathic" electromyogram (EMG) might in several instances be caused by abnormality of the lower motor neuron or its axonal branches, and thus it would actually be "pseudomyopathic". Examples are myasthenia gravis, central core disease, and myotonic atrophy (formerly called myotonic dystrophy).

Detailed and repeated electrophysiologic measurements on patients treated with our newly-introduced program of long-term high-single-dose alternate-day prednisone clearly documented both a 48 hour cyclic and more sustained cumulative benefit of prednisone on muscle strength and endurance. In one patient the improvement in adductor pollicis action potential response to repetitive nerve stimulation during the evening of on-prednisone days, as compared with off-prednisone days, indicated that maximal benefit occurred during the prednisone day. In another patient, comparison of the amplitudes of the 2 orbicularis oris responses to twin shocks to the facial nerve delivered at intervals of from 10msec to 1,000msec, demonstrated increased synaptic efficiency at each inter-shock interval during the evening of the prednisone-treatment day as compared to that morning before receiving prednisone. Both effects were best demonstrated during the initial days of treatment of a third patient. Lessening fall-off of amplitude of abductor digiti quinti response to twin ulnar nerve supramaximal shocks 300msec apart showed that improved neuromuscular synaptic efficiency could be detected within several hours, reached its peak at around 12 hours, and then ebbed to a level of overall improved function until a succeeding 48 hour cycle of improvement followed the next prednisone dose. The electrical improvement correlated directly with improved clinical strength in this and other involved muscles. This 48 hour cycle, being in phase with prednisone administration but not with any other medication or environmental variable, is convincing evidence that prednisone therapy is responsible for improvement. During the early stage of treatment, strength in involved muscles during both the "peak" and "ebb" phases of each 48 hour cycle was greater than during the preceding 48 hour cycle, resulting in a continued net improvement. Later the rate of improvement was more gradual. (Publications recorded under our Myasthenia Gravis Project).

Electrophysiologic studies, combined with histochemical and clinical studies, also allowed theoretical formulation of the basis of the clinical improvement in myasthenia gravis with high-single-dose alternate-day prednisone, as follows: rapid improvement may represent correction of a defect in neuronal axoplasmic flow, which might initially improve the defect of motor nerve ending excitatory function. Intermediate improvement may reflect physical repair of nerve endings and the post-synaptic region of the myofiber. Later improvement may be based on return toward normal of atrophied myofibers. (Publications recorded under our Myasthenia Gravis Project).

In an increasing number of chronic neuropathies studied, action potentials of greatly enlarged size, but not increased firing frequency or altered rhythmicity may be the first recruited, to be followed by recruitment of action potentials of successively greater size. Whether this is due to retention of the inherent soma-dendritic properties that determine recruitment order, despite reorganization of the peripheral parts of the motor unit, or represents loss of reorganization of the peripheral parts of the motor unit, or represents loss of presumably smaller motor units, is being analysed. Similar studies are being extended to myopathies.

Normal standards for voluntary action potentials and nerve conduction studies for this laboratory are being collected.

Significance to Bio-Medical Research and the Program of the Institute: Treatment of human neuromuscular diseases is based on precise diagnosis and understanding of the pathophysiology. As a group, human diseases of the lower motor neurons (including peripheral neuropathies) and muscle are just beginning to have their pathophysiology partly understood. The detailed neurophysiologic techniques herein described are leading to more precise delineation of these diseases, and in some instances causing us to completely change our concepts of their pathogenesis.

We are also now learning about different physiological properties of the various types of human motor units. These are being correlated with histochemical studies and compared with analysis of motor unit types in the cat, using techniques that are not possible in humans (see our Histochemistry Project).

The use of electrophysiologic criteria to support clinical observations of evaluating our new program of long-term high-single-dose alternate-day prednisone in myashtenia gravis has been extremely useful in our concluding, tentatively, because of our limited number of cases to date, that this now seems to be the best treatment available in myasthenia.

Proposed Course of Project: This project will be continued and elaborated upon, e.g., with even more detailed correlation between EMG and histochemistry in patients and further studies in myasthenia gravis.

Honors and Awards: None

Publications:

Warmolts, J. R. and Engel, W. K.: A critique of the "myopathic" electromyogram. Trans. Amer. Neurol. Ass. 95: 173-177, 1970.

Warmolts, J. R. and Engel, W. K.: Correlation of motor unit behavior with histochemical and myofiber type in human by open-biopsy electromyography. Intern. Cong. of Electromyography, Sept. 12-15, 1971, Brussels. In press.

Warmolts, J. R. and Engel, W. K.: Correlation of motor-unit behavior with histochemical myofiber type by open-biopsy electromyography. Trans. Amer. Neurol. Ass. In press.

Serial No. NDS(I)-62 MN/AP 926(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.9
Other	0.0

Project Description:

Objectives: The sequential pattern of physiological events which develop in skeletal muscle starting with excitation and ending in relaxation has not been adequately defined. The molecular interactions that link excitation to contraction in the physiological state are largely unknown. Not much knowledge has accumulated about the molecular changes involved in the uncoupling of contraction from excitation which permits relaxation. The objective was to establish an ionic basis for initiation, maintenance and subsequent loss of tension in slow-type skeletal and in smooth muscle. An additional objective was to relate the ionic events to electrical, mechanical and metabolic activity and to determine how these parameters were related to drug action.

Methods Employed: The coupling and uncoupling processes between the tension-relaxation cycle and the related ionic events are investigated in smooth muscle and in slow-type skeletal muscle. Slow skeletal muscle has now been found to occur in many mammalian species and smooth muscle occurs in all mammals. Since classical electrophysiological and mechanical methods have failed to explain the coupling process ion substitution methods have been developed and used. Sucrose gap techniques have been modified and used for monitoring membrane potential changes. Ion contents are measured by atomic absorption spectrophotometry. Di-monovalent di-buffer amines have been developed and used as monovalent, nonpenetrant, and electrical charge substitutes for Na^+ and Cl^-.

Major Findings: The study of the ionic basis for the tension relaxation cycle of events has been continued using smooth mammalian muscle. Smooth muscle contraction brought on by a change in external ions can be myogenic since drugs that block both neurohumoral agonists and nerve impulses do not affect contraction. The kinetics of ion effects, active transport inhibition by a drug, shortening or relaxation are greatly accelerated in isolated smooth muscle as compared to slow skeletal muscle treated similarly. The contractile responses of smooth muscle resembles that of slow skeletal muscle in that the removal of any one of physiological ions from the external environment can induce tension or contraction. The contraction of smooth muscle after the removal of external K^+ is rapid and easily reversible by return of the ion. Since external K^+ activates transport ATPase, smooth muscle may be kept in a relaxed state by the ATPase transport systems. Inhibition of ATPase by ouabain also induces contraction in smooth muscles that is followed by full relaxation. A relaxed muscle but one fully inhibited by ouabain fails to contract in response to complete removal of external K^+. These findings are in agreement with the concept that an electrogenic pump is of physiological importance in smooth muscle. Factors other than ion transport are of importance in the initiation of contraction since substances such as valinomycin which increase K^+ permeability of membranes block contraction brought on by K^+ depletion. Drugs such as adrenaline which hyperpolarize and prevent spontaneous activity of smooth muscle only partially block contraction induced by K^+ withdrawal.

Significance to Bio-Medical Research and the Program of the Institute: The research of this project continues to furnish new data of a fundamental nature related to muscle malfunction. Knowledge about smooth muscle may eventually help explain whether vascular smooth muscle is involved in stroke mechanisms or the etiology of "Duchenne's" dystrophy.

Proposed Course of Project: The work of the project should continue as long as it yields new knowledge about a tissue system in which disease processes occur that are not fully understood. More data on ion conductance, membrane properties and contraction will be obtained on smooth muscle. New test systems using vascular smooth muscle can be developed.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1049(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: Melvyn N. Kronick
George A. Simon

Cooperating Units: Biomedical Engineering and - Instrumentation Branch
Division of Research Services

Man Years

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: (1) General - Implementation of new methods which permit investigation of contractile properties of active skeletal muscle and their modification by drugs are currently in progress. (2) Specific - In cooperation with the Biomedical Engineering and Instrumentation Branch we have proceeded, for the past year, to design, fabricate and test an apparatus which compliments our investigation of the biomechanical properties of active skeletal muscle. Incorporated into the apparatus are current state-of-the-art control systems which provide the necessary response time not previously achieved with instruments used for similar purposes.

Methods Employed: The apparatus is basically a voice coil displacement transducer, controlled by electric current, which is operated in a feedback configuration. Positive or negative displacements (stretches or releases respectively) were applied to isolated active amphilian muscle sufficiently fast to permit separation of the slowly responding contractile element from the more rapid elastic component.

Major Findings: The specific goal was a reinvestigation of the rise time of activity in the muscle after initiation of stimulation. Muscle stiffness, one indicator of contractile activity, was assessed from the amplitude of tension spikes produced by brief short stretches applied to the muscle via the apparatus. Contractile activity subsequent to stimulation rose in a finite time. The rate of rise was estimated to be 40-60 P_o/sec at room temperature. The rate was also found to be temperature dependent with an estimated Q_{10} of two. A preliminary account of the apparatus was presented to the 23rd Annual Conference on Engineering in Medicine and Biology in November, 1970; and the results outlined above were presented to the 15th Annual Bio-physical Society Meeting in February, 1971.

Significance to Bio-Medical Research and the Program of the Institute: The mechanical characteristics and contractile properties of active muscle serve as the standard for evaluation of the effects of disease, environment, drugs, etc., on contractile responses. The capability of estimating the time course of activity following stimulation is of particular significance in the assessment of certain drug actions which are known to influence the contractile responses of voluntary muscle.

Proposed Course of Project: A more detailed description of the response of active muscle to such brief stretches is planned. The effects of certain chemical agents on the time course of muscle stiffness as well as extension of these observations to mammalian muscle is also anticipated.

Honors and Awards: None

Publications:

Kronick, M.N.; Simon, G. A. and Wells, J.B.: Transducer for studies of active muscle. Proceedings of the 23rd Annual Conference on Engineering in Medicine and Biology. Biomechanics. 12: 63, 1970.

Serial No. NDS(I)-63 MN/AP 1050(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Functional Activity and Mechanical Properties of Striated Muscle

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: (1) General - The investigation of adaptive and trophic mechanisms in living striated muscle is approached in this laboratory through analysis of mechanical properties and responses of the active muscle. Thus, mechanical measurements which are applied to the various mammalian species available, are expected to reflect such trophic and adaptive mechanisms thought to underlie the rather wide spectrum of contractile responses observed. (2) Specific - The relationship between characteristic speed of contraction and the fiber type (histochemical) composition of the muscle is currently under investigation. Specifically, our current observations were made on animals at several stages of maturation during which time muscle histochemistry has been observed to change.

Methods Employed: Contractile responses were recorded *in situ* under isotonic and isometric conditions. A rate index previously established in this laboratory as an indicator of muscle speed was derived from the isotonic data to assess characteristic contractile velocity in new born kittens.

Major Findings: Because of the "seasonal" nature of the availability of new-born kittens, namely April through June, the measurements were taken and necessarily put aside for subsequent processing and evaluation. Responses from anterior tibial muscles of 8 kittens 18 - 100 hours old, which are currently being evaluated, will be compared with a similar series of soleus muscles from the same aged animals to determine if a characteristic contractile speed has been established at this developmental stage. A velocity difference observed between these representative muscles at this age would strongly suggest basic differences in the contractile material of each muscle type despite their similar histochemical appearance.

Significance to Bio-Medical Research and the Program of the Institute: The work outlined above was designed to contribute information concerning a possible relationship between the contractile character of skeletal muscle and its histochemical activity. Such information would be useful in the clinical interpretation of histochemical changes which occur in certain myopathies.

Proposed Course of Project: It is planned to expand such measurements to mammalian species which show natural adaptations in their muscular or locomotor activity.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 MN/AP 1416(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.
Ahmed Hassan Mohamed, Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt

Man Years

Total:	0.1
Professional:	0.1
Other:	0

Project Description:

Objectives: To obtain and investigate heretofore unavailable venomous substances. Attempts are being made to first determine the primary point of biologic activity and then to find out the tissue and cellular mechanisms of neurotoxic activity.

Methods Employed: The above objectives demand the use of a wide variety of whole animal, isolated tissue, and cellular techniques involving toxicological, pharmacological, and biochemical procedures. One or two well-known venoms in each genus are investigated in order to establish a standard. A comparison of the activity of the venoms from the more rare species aids the discovery of new properties of the various venoms with a minimum of search.

Major Findings: One of the most troublesome problems in neurologically oriented research with venoms is the lack of uniformity of the test samples because of variation in source of supply, collection, process and storage. Geographical location has been identified as one variable of venom source. Standardization of certain Elapidae venoms has been accomplished and reported. The sequence of biological activity for the venoms categorized as neurotoxic progresses from an initial paralysis of peripheral origin to cardiovascular toxicity and eventually to CNS effects. Intra-arterially applied neurotoxic venoms damage the blood-brain barrier sufficiently to permit entry of large dye molecules into the brain.

Significance to Bio-Medical Research and the Program of the Institute: Past investigations of venoms have been preeminently successful in various aspects of biology.

The collection of venoms from poisonous species has made possible the scientific development of antivenins for the treatment of envenomation by poisonous animals. The research here is collaborative with and supportive of a program that is now designed to collect venoms from the entire African Continent (except for the Union of South Africa) and to develop polyvalent antivenom for therapeutic use.

Because of the accelerating toxicity to which modern man is exposed, the study of toxic and poisonous substances becomes of increasing importance. Only a few relatively common venoms have been investigated. Over 600 different species of poisonous snakes are believed to exist in the world. What wealth of biological tools are undiscovered in the venom of the lesser known species can only be surmized from the success of past research. The relationships among snake venom neurotoxins, enzymes, antivenins, toxicities, and therapeutics are almost completely unknown. The project, therefore, has excellent possibilities of increasing progess in medical research.

Proposed Course of Project: The collaborative effort which makes this project possible has been funded for the second year of the extension by the PL-480 program. Because of the war atmosphere of the collaborative program location and its requirements on the principal investigator communication and progress has been slow and difficult. For the same reason the future of the project remains uncertain.

Honors and Awards: None

Publications:

Irwin, Richard L., Oliver, K. L., Mohamed, A. H. and Haast W. E.: Toxicity of elapidae venoms and an observation in relation to geographical location. Toxicon. 8: 51-54, 1970.

Serial No. NDS(I)-70 MN/AP 1809(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Impaired Transfer of Information Between Nerve and Muscle

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Jay B. Wells, Ph.D.
Katharine L. Oliver, B.S.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.9
Other:	0

Project Description:

Objectives: To find what biochemical deficiencies impair transfer of biological information between nerve and muscle.

Methods Employed: Suitable animals are made deficient in a functional component of a metabolic pathway related to transfer of information between nerve and muscle by either diet or chemical inhibitors. Loss of transmission efficiency is studied in vivo by use of a dual channel digital data acquisition system. Restoration or failure of function is determined by return of the metabolic components or precursors to the test animal. Simultaneous comparisons are made between muscle types.

Major Findings: Adult rats kept for many weeks on a choline-free diet with access to their total environment practice coprophagy, recycle choline and transfer information between nerve and muscle in a normal way. Weanling rats denied choline became metabolically impaired as indicated by death of some animals, loss of hair, eye and kidney hemorrhage, cataracts, large fatty livers and slow growth rates. When tested these severely choline deficient rats maintained a nearly normal ability to transmit signals between nerve and muscle. This indicates that even under severe depletion of choline the nervous system can utilize or synthesize choline to a greater degree than some other organ systems or that motor nerves may have a low choline requirement. It is of general interest to the neurological sciences that animals with major metabolic defects in many organ systems because of choline lack appear to have no deficiency in the nervous system, or at least in that part we have tested.

Significance to Bio-Medical Research and the Program of the Institute: There is a great need for increased knowledge of malfunctioning muscle in neuromuscular diseases. The project is designed to furnish data that will aid in the understanding of the biochemical processes involved in muscle that fails to function.

Proposed Course of Project: This project is to be continued until biochemical deficiencies that impair transmission of information between nerve and muscle have been identified.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Branch of Surgical Neurology, IR
National Institute of Neurological Diseases and Stroke

J. M. Van Buren, M. D., Acting Chief

In the proposed reorganization of SN of September 10, 1970 the work of the Branch is divided into:

(1) Studies of treatment of disease of the human CNS
(2) Studies of the form and function of the human CNS

This dichotomy of interest in the Branch is dictated by the opportunities for study not only of the response of man to disease of the CNS and its treatment but of the basic form and function of the human nervous system during neurosurgery. In many instances these opportunities far surpass what can be achieved under the best experimental situation in lower primates. The varied skills of Branch personnel support the concept of multifaceted studies of each patient which reap the maximum benefit from money spent in ward care.

The Section of Child Neurology represents an essentially separate and autonomous area in SN. For this reason, the summary of the activities of this section are reported separately.

I. Tumors of the CNS (Clinical Brain Tumor Program)

Basic Studies:

A. Diagnostic methods: Radioisotope scanning detection and localization of endocranial and intraspinal lesions has received continued emphasis. The dog has provided an experimental model and the normal scan pattern has been recognized. Studies in dogs of experimental hydrocephalus, tumors and hemorrhagic lesions are in progress. Special interest has been taken in the possibility that anti-tumor materials tagged with radioactive compounds would reveal the concentration gradients of these substances in the CNS. This would provide not only a more rational approach to chemotherapy but also an objective and quantifiable index of tumor response.

B. The achievement of a functional tomoscanner in the past year, though briefly stated, is a matter of major importance since it adds a new dimension in scanning precision.

C. Studies of normal characteristics (resting membrane potentials, resistance, capacitance, time constant) of both excitable (neurones) and non-excitable (? glia) cells have paved the way for application of these techniques to tumor material both in vivo and in vitro. This would permit evaluation of the differential effects of chemotherapeutic agents on normal and neoplastic components of the brain.

Clinical Studies:

A. A special device has been developed to permit intratumoral instillation of lipid soluble chemotherapeutic agents by percutaneous puncture. Combined with the isotope techniques outlined above it offers a wide field for study. Withdrawal of fluid from the artificial tumor cyst will permit search for specific tumor antigens. This could pave the way for specific immunotherapeutic methods of prevention and treatment of glial tumors.

II. Vascular disease of the CNS (Clinical spinal AVM program)

A. Spinal vascular disease

Basic studies:

1. Experimental angiography in the monkey with use of abdominal compression and pressor substances has demonstrated the normal spinal veins and thereby the angiographic circulation time of the normal cord. A complex pattern of ascending and descending blood currents has been found. A model for spinal vascular occlusive disease in the monkey (with autopolymerizing silicone rubber immobilization) is being investigated. It is hoped to apply these new findings to human disease of the spinal cord.

Clinical studies:

1. The value of the initial classification of AVM (type I, single coiled vessel; type II, glomus; type III, juvenile) has been confirmed by continued experience and each type has been found to respond best to specific measures. A collaborative study, offering a large quantity of clinical material, is planned in India under PL-480.

2. In a continuing study of human spinal angiograms (50 to date) the characteristic features of hemangioblastoma, AVMs and intradural extramedullary tumors were studied. An important and possible essential association of aneurysms with bleeding from a spinal AVM has been noted. Studies have shown new criteria of obstructive vascular disease of the spinal cord.

B. Cerebral vascular disease

Basic studies:

1. Using a monkey model studies of occlusive disease have been started with selective ligation of the sagittal sinus at various levels.

2. Completion of studies of the extracellular space and the normal electrical parameters of excitable and nonexcitable units in the cerebral cortex permit extension of these techniques to study of brain edema and the effects of vascular changes.

Clinical studies:

1. Studies are under way to evaluate the efficiency of arterial crossover in patients with essentially normal cerebral vasculature. This will provide data regarding avenues of collateral flow of possible importance in the susceptibility or resistance to stroke.

2. Review of the significance of intimal versus medial calcification of the carotid siphon in elderly males has shown a differing prognostic significance. The former has a diminished cerebral blood flow values of 52.1 ml/100 gm/min while the latter has normal values of about 60 ml/100 gm/min.

III. Dyskinesias (Clinical stereotaxic program)

Basic studies:

A. Neurophysiology

Extrapyramidal and sensory control of motor function have received experimental study. There is evidence of afferent fibers in the motor cortex from various subcortical basal nuclei. The cerebellar nuclei influence the function of the basal nuclear structures (dentate) including the red nucleus (fastigial nucleus). Further experiments will investigate these nuclei following lesions and chemoinjection.

Support has been found of earlier evidence of a "motor correlative discharge" producing sufficient feedback information for motor control in upper limb deafferentiation experiments in monkey. The caudate nucleus was important in this mechanism. This correlated with the finding in chimpanzee that there were discrete caudate projections from the precentral gyrus in anatomical studies.

Human neurophysiological studies with simultaneous recording from two microelectrodes showed that some cells of the motor cortex had a close firing relationship to motor movement (e.g., Parkinsonian tremor, voluntary flexion or extension of the opposite extremity). There was also evidence that the activity of some cortical neurons is dependent upon the activity of the thalamic neurones. The analysis of the data, with a more sophisticated electronic instrument is in process.

B. Neuroanatomy

A long term project has been completed this year which, for the first time, defined the cytoarchitectonics of the human thalamic nuclei by Golgi as well as usual techniques and defined the cerebrothalamic relationships in a large series (54) of human hemispheres. In addition, the variations of the thalamic (15 specimens) and diencephalic-mesencephalic structures (75 specimens) to the surgical stereotaxic coordinates have been studied in three planes. This work is basic to the evaluation of electrode position from X-ray landmarks in man.

Clinical studies:

A. With the advent of L-dopa in the treatment of Parkinsonism it became important to evaluate the long term results of thalamotomy in the treatment of this disease to see what the relationships of these two methods of treatment would be. Eighty-nine Parkinsonians treated by unilateral thalamotomy and followed 3 to 10 years with a variety of qualitative and quantitative tests of motor function were evaluated. Among the many findings, tremor was found to respond most favorably and continued to improve on the average over many months following operation with a peak about 3 years following surgery. After this the progressive nature of the disease took its toll. Rigidity responded less well and bradykinesia and midline symptoms not at all. Initial experience with L-dopa suggests that its best effect is on bradykinesia and rigidity and not tremor so that the best therapeutic effects will be achieved by a combination of the drug and surgery.

IV. Effects of trauma on the CNS (No clinical material)

Basic studies:

A. Mechanics

Studies of the mechanics of head injury indicate the importance of rotational versus pure linear displacement in producing measurable brain distortion. The rotation frequency of brain distortion = 5-10 Hz.

B. Model and scaling factors

Special studies have been undertaken to develop an experimental model for CNS trauma with scaling factors to permit application of quantitative data of predictive value in man.

The degree of unconsciousness produced by trauma and electroshock was compared in mice and found sufficiently different in character so that ECT did not offer a satisfactory trauma model despite earlier evidence to the contrary.

Sensory evoked response methods have been worked out in the awake chimpanzee and will help in evaluation of CNS trauma in this animal.

Data testing of the lumped parameter model for head injury has been started and this model will soon be ready for predictive experimental validation.

Clinical Studies:

A. The ability to define an injury threshold for cerebral concussion and other effects of CNS trauma in man provides the possibility of more adequate performance criteria for brain protection during head injury and whiplash. From a practical standpoint, a cervical support increases the threshold for experimental concussion in the monkey. Experimental whiplash (alone) produces visible brain surface hemorrhages.

Postmortem studies of two human whiplash cases have given support to the present scaling criteria.

V. Speech, memory and cognitative function in man (Surgical epileptic and stereotaxic clinical programs)

A. Memory

Stimulation in the cingulum produced severe impairment in recall for verbal memoranda from the left but not the right side. This differed from the language difficulties from left thalamic stimulation. After bilateral cingulomotomies with clinical improvement there were not comparable deficits in memory and intellectual achievements. This adds to the evidence of the role of the cingulum as a modulator of emotional responsiveness implicating more distal limbic mechanisms in memory functions.

Stimulation of the posterior temporoparietal cortex produced interruption of rote name memorization even in the absence of dysphasia or perceptual faults. Evidence of differing memory localizations appeared. Stimulation of the anterolateral temporal cortex interfered with short term or buffer memory while not producing dysphasia. Long term memory storage appeared more related to the posterior temporoparietal system which is least dispensable for receptive and expressive language.

B. Speech

Quantitative studies of speech production during amytal irrigation of the hemisphere showed a longer lasting deficit in recovery of basic linguistic skills and longer lasting deficit in immediate verbal memory in the dominant hemisphere. A hierarchy of effect became apparent with sparing of basic neural speech mechanisms regardless of side of amytal injection. At an intermediate level, recovery of speech and test orientation could precede recovery of ability to name simple objects. Finally, even after recovery from dysphasia, short term verbal memory might remain impaired for objects correctly named 12-15 seconds earlier.

Special study showed no influence of initial and final phonemes in naming errors during amytal tests. The relationship of naming difficulties and the phonemic structure of test words is being studied during disruptive stimulation both of depth and surface structures and during the carotid amytal test.

C. Cognitive function

Evoked potentials recorded from implanted and scalp electrodes differed with perceptual and nonsense material more in the left (dominant) than in the right hemisphere regardless of the position of presentation in the visual field. The asymmetry suggested that there is favored reception of the material in the left hemisphere regardless of the side of initial reception in the brain. Left temporal lobectomy resulted in undifferentiated wave forms associated with verbal material and the same was seen with nonverbal material (nonsense dot patterns) after right temporal removals.

Report of the Section on Child Neurology

The program of investigation falls into three main categories:

I. Clinical, structural and biochemical studies of metabolic diseases affecting the nervous system.

Determination of all lipid classes and mucopolysaccharides (MPS) in the brain and other organs of late infantile G_{M1}-gangliosidosis confirmed elevation of G_{M1} but disproved claims of increase in MPS. Demonstration that β-galactosidase activity in the white cells of heterozygous carriers is decreased by 50% will permit premarital counselling of siblings and relatives.

In contrast to Hunter's and Hurler's patients, those belonging to Sanfilippo's type mucopolysaccharidosis had severe degeneration of neurons in layers 4 and 2 (intellectual and associative function) while layers 5 and 3 (efferents) were well preserved. This finding helps to explain early mental deterioration with good motor function in Sanfilippo's syndrome.

The technique developed by the Section demonstrating molecular weight distribution of MPS proved a useful tool in assessing therapeutic responses in mucopolysaccharidoses and cystic fibrosis.

II. Epilepsy during early childhood; clinical and biochemical studies.

Understanding of the mechanism of ketogenic diet in the therapy of epilepsy was advanced by experimental study. Weanling rats fed high fat diet for three weeks showed increase in content of cholesterol and phospholipids in the synaptic vesicles and nerve endings. This finding may be related to the increased threshold of excitability.

Clinical studies of the therapeutic action of the ketogenic diet continue.

III. Brain development and conditions underlying cerebral palsy and mental retardation; clinical, developmental, pathological and biochemical study.

A comprehensive study, "Malformations of the Nervous System: Medical Aspects" is in press relating to (a) the prenatal development of the human brain, (b) pathological features of the congenital malformations of the CNS, and (c) determinations of the teratogenetic periods of various malformations. These basic data provide clues regarding the etiology and pathogenesis and a guide to the long range management of some of these conditions.

Serial No. NDS(I) - 54 SN/OC 100(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M. D., A. K. Ommaya, F.R.C.S. and C. Ajmone-Marsan, M. D.

Other Investigators: P. Fedio, Ph.D. and H. Lansdell, Ph.D.

Cooperating Units: N-EEG

Man Years
Total: 1.6
Professional: 1.2
Other: 0.4

Project Description:

Objectives:

1. To study causal mechanisms of epileptic seizures in man and other primates.

2. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.

3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical neurological examination.

2. Special radiographic and other contrast examinations.

3. Electrographic, including electrocorticographic, examination.

4. Physiological and psychological techniques as indicated.

5. Histological and chemical examinations as required.

Major Findings:

1. The surgical material has provided brain tissue for special neurochemical studies which are reported elsewhere (Serial No. NDS(I) - 69 SN/CN 1655).

2. Stimulation of implanted depth electrodes at open craniotomy have provided material for study of speech, memory and cognitive functions which are reported elsewhere (Serial No. NDS(I) - 57 SN/CP 401(c) and NDS(I) - 68 SN/OC 1658(c)).

3. A special study by Dr. Cosimo Ajmone-Marsan is presently being undertaken of the criteria for operation of patients with bitemporal epilepsy based upon data derived from scalp and depth electroencephalograms (Serial No. NDS(I) - 61 EEG/OC 800(c)).

4. Work on the late follow-up of the results of epileptic incisions was started with temporary personnel during the summer of 1970. Due to the loss of this personnel, the study had to be temporarily terminated.

Significance to Bio-Medical Research and Program of the Institute: This project attempts further understanding of epileptic mechanisms, with particular reference to therapeutic applications, and the exploitation of the surgical treatment of these conditions for cortical physiology and for cerebral physiology and behavioral mechanisms.

Proposed Course of Project: The collection of data for the various studies mentioned above will continue. It is hoped that personnel can be brought on to continue the important studies of the late follow-up results of epileptic excision.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 55 SN/OC 200(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Involuntary Movements

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M. D., Choh-luh Li, M. D. and Paul Fedio, Ph.D.

Other Investigators: D. Y. Shapiro, M. D., W. G. Henderson, Ph.D. and D. Sadowsky, B. S.

Cooperating Units: N-OB

Man Years
Total: 2.4
Professional: 2.1
Other: 0.3

Project Description:

Objectives: Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed: Surface and depth microelectrode recording of the human brain makes use of specially built high impedance pre-amplifiers, tape recording of signals and eventually averaging and cross correlation of cellular activity and other functions such as muscle activity and brain potential variations from large electrodes. Depth electrode stimulation employs a specially built electrode and a current monitored stimulator. The special apparatus used in quantitative psychological testing is described in Dr. Lansdell's and Dr. Fedio's Project Report Serial No. NDS(I) - 63 SN/CP 1033(c).

Evaluation of the postoperative Parkinsonian patients was based on data derived from four sources: (1) history; (2) physical examination; (3) specific psychological tests (Wechsler Adult Intelligence Test, Wechsler Memory Test and MMPI); and (4) battery of clinical quantitative neurological tests administered in part by the examining physician, in part by the physical therapist and in part by the occupational therapist. Due to the patient recruitment from wide areas, current patient review has not been finished and the present final patient sampling is smaller than it will eventually be. This makes data analysis more complex at the moment but the use of special mathematical tools permits even at this time statistically reliable statements about the long term beneficial results of thalamotomy.

Major Findings:

1. The 40 odd tapes of extracellular unit recordings in the human cortex and basal ganglia have been under analysis by Dr. Li for some time and will be presented in his Annual Report Serial No. NDS(I) - 62 SN/OC 913(c).

2. The effects of depth stimulation upon speech and cognitive function have been studied in detail by Dr. Paul Fedio and the results will appear in his Annual Report Serial No. NDS(I) - 66 SN/OC 1245(c).

3. The late follow-up of Parkinsonians subjected to unilateral thalamotomy was particularly pertinent at this time due to the advent of L-dopa in the treatment of Parkinsonism. Although many studies of the early effects of thalamotomy had appeared and had received considerable attention due to their spectacular nature, late studies are infrequent. In addition, all the late studies to date have been anecdotal in nature without the application of quantitative types of neurological motor testings, nor have they made use of more sophisticated mathematical techniques such as multifactorial analysis which can indicate the statistical reliability of the test results.

A total of 87 patients were treated with unilateral thalamotomy over the ten-year period 1960 - 1970 for Parkinson's disease. At the present time 61 of these have been analyzed.

The quantitative data were analyzed in three ways. Firstly, for each test in each examination period a mean score was calculated. Since the sample of patients for each test and each examination period was somewhat different from the other sample of patients for any other test and other examination period, the mean score of the test in any postoperative period could only be compared with the mean preoperative score of the same sample of patients. Hence, each postoperative mean score of each test had its own preoperative mean score with which it was compared. Thus, although patient samples differed from test to test and examination period to examination period, meaningful comparisons could always be made between the mean scores of each postoperative sample and the mean of its own preoperative scores. In other words, each group of patients served as its own standard of measurement. A paired t-test was applied to test the statistical significance of the difference in the pre- and postoperative means.

Secondly, the postoperative-preoperative difference in test score, for each individual patient, for each test and each examination period, was marked "better" if the difference was equal to or greater than 20% improvement. It was marked "worse" if the difference was equal to or greater than a 20% worsening. It was marked "unchanged" if the difference fell between 20% improvement and 20% worsening. The number of patients who improved was summed as was the number of patients who worsened. A two-tailed sign test was applied to test for the statistical likelihood that this number of "better" or "worsened" patients could occur by chance. Again each patient sample served as its own standard of measurement.

Thirdly, normal volunteers were calculated for each test by testing a group of 32 normal volunteers with age distribution similar to that of the inpatient sample. Each individual preoperative and postoperative test score was then converted to a percentage of normal. Converting all scores to per cents of normal permits aggregating scores of tests measuring similar neurological function. This is done so that combined results of all quantitative tests, speech tests, strength tests, fine manipulation tests, gross manipulation tests and ambulation tests could be presented.

Tremor:

(1) Tremor on the non-operated side: Through the fourth postoperative year some 65% of the patients showed definite tremor improvement whereas only 15% showed initial improvement and then progressive deterioration. The quantitative data showed: (a) a drop in the number of patients with demonstrable tremor significant at 0.025 probability level through the fifth postoperative year; (b) the percentage of patients free from tremor rose from 0 to a maximum of 84% in the third postoperative year, thereafter falling to 58% in the fourth year; (c) the mean amplitude of tremor during finger apposition was below preoperative amplitude persistently through the third year at a significance level of 0.05; (d) mean tremor amplitude during finger apposition decreased by 16% at three months and by 100% at three years; and (e) duration at which tremor could be suppressed in finger apposition increased 183% in three months and returned to preoperative levels by 12 months.

(2) Tremor on the same side as operation: The ipsilateral effect of operation on tremor was considered as an index of the patient's overall disease. Less than 5% of patients showed improvement in tremor on the same side as surgery. The per cent of worsening of tremor rose steadily from 15% at three months to 67% at four years. An unexplained reduction of severity in tremor appeared transiently on the ipsilateral side following thalamotomy.

Rigidity:

(1) Rigidity on the non-operated side: Rigidity was improved initially in 72% of the patients, but by the third year as many patients were worsening as were better than preoperatively. The magnitude of improvement as measured by the wrist flop was 50%.

(2) Rigidity on the same side as operation: The per cent of patients displaying rigidity on the operated side increased to 25% by the fourth year. Otherwise the symptom changed very little.

Bradykinesia:

Although the qualitative data showed a steady increase over four years in the per cent of patients worse in overall bradykinesia, the quantitative data showed a three year 30% improvement in the tapping speeds of finger and ankle on the non-operated side. Tapping speeds of all other joints of both sides did not change.

Speech:

Qualitative data showed a slow increase in the per cent of patients worse in voice volume, speech clarity and voice production from 8% at three months to 40% at six years. No quantitative data was taken to measure the magnitude of this change.

Mental Status:

Qualitative data showed only a very gradual increase in the per cent of patients worse mentally postoperatively than preoperatively from 3% to 15% over four years. The quantitative data, the Wechsler Intelligence and Memory Test and the MMPI, will be evaluated in a separate report. The test called cognitive showed little change in mean magnitude of performance for three years.

Combined Symptoms:

When all symptoms other than rigidity and tremor on the non-operated side were considered, the per cent of patients worse than preoperatively increased rapidly until at four years 80% of the patients were worse in at least one of these symptoms.

General Function:

Qualitative data showed that although 67% of the patients were functionally better immediately postoperatively, by the third year the rising per cent of functionally worse patients equalled the falling per cent of patients still functionally better. Quantitative data showed that in no patient sample was the mean improvement better than 25%. (This should not be interpreted to mean that no individual improvement was more than 25%.)

Of the series, one patient died of thalamic brain stem hemorrhage. Careful testing indicated some reduction in performance in 35 (63%) of the patients following surgery. Fourteen patients had mental changes postoperatively, an incidence of 25%. Fifteen patients had local sensory changes in an incidence of 27%. Seventeen patients had some degree of hemiparesis

(29%) and nineteen had a coordination deficit (an incidence of 34%). It should be pointed out that nearly all patients showing sensory motor deficits to careful quantitative evaluation were considered "good" results on the Ward and both the patient and the patient's family agreed with this evaluation. The vast majority of complications were transient and disappeared within the first postoperative year. On the whole, operative complications were not factors limiting the beneficial effects of first thalamotomy.

One of the most interesting features was the discovery that tremor opposite to the side of operation, although it improves immediately postoperatively, actually continues to improve for three years both qualitatively and quantitatively in terms of the tremor amplitude itself. This slow, continuous improvement following surgery suggests that the thalamotomy sets in motion a secondary neuronal degeneration process which requires certain time in order to interfere with the primary tremorogenic mechanisms.

Significance to Bio-Medical Research and the Program of the Institute: The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible even with the time consuming conditioning experiments applied in lower animals. The use of the human subject is therefore not a duplication of animal experimentation but an extension of this and, of course, studies of speech function may only be carried out in man.

Proposed Course of the Project: Continued efforts have been made to enlist engineering skills to attack more directly the problem of the pathophysiology of motor activity itself. As yet it has not been possible to attach to the Branch a person with adequate engineering and computer background to devote sufficient time to work in collaboration with the clinical service. The present evaluation of the postoperative effects of thalamotomy have made of use some very primitive testing procedures evaluated by a sophisticated and well controlled method presented by Dr. Henderson. Return to more sophisticated types of motor testing, however, merits the detailing of engineering personnel interested in the problem.

In view of the hint that secondary degeneration may be responsible for the good effects of thalamotomy on tremor, request has been made for an additional person to undertake the anatomical study of some ten serially sectioned human brains which were obtained one to several years following thalamotomy. Detailed reconstruction studies of the thalamus, basal ganglia and brain stem hopefully may give a lead as to the mechanisms of therapy.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Previous Serial Number: Same

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: R. C. Borke, B. S. and R. Schilling, R. EEG T.

Cooperating Units: N-EEG

Man Years

Total:	2.4
Professional:	0.3
Other:	2.1

Project Description:

Objective: This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

1. Serial sections of human and animal brains in celloidin for myelin and Nissl's series.

2. Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

In the past year the major anatomical work has been finished which occupied nearly the full-time research efforts of the principal investigator for the last three years. The first volume, entitled THE NUCLEI AND CEREBRAL CONNECTIONS OF THE HUMAN THALAMUS, contains the characterization of the human thalamic nuclei worked out from original material both in Golgi and chromatic stains. A major effort has been made to obtain all pertinent thalamic references in the three major languages and particular attention has been paid to the studies of thalamic connections.

Using 54 serially sectioned human specimens with long standing focal lesions of the cerebrum, the cerebral and thalamic connections have been worked out in detail for the first time in man. Since often the natural lesions occur in positions which are not surgically accessible in the experimental animal, this has provided a considerable amount of new material. Quite obviously due to the length of the text it cannot be incorporated here. However, the following example may be given:

Connections of N. Pulvinaris Medialis in Man

> Various investigators (Clark and Northfield, 1937; Walker, 1938; Locke, 1960) have indicated that the lateral convexity of the posterior parietal and temporal cortices in primates is the cortical projection field of the medial division of the pulvinar (Pu m). However, studies of eight hemispheres of serially sectioned human brains provide substantial evidence to relate Pu m to lesions involving the medial portion of the parietal, temporal and probably anterior occipital regions. Furthermore, a scheme of topographical representation can be derived from the application of the cortico-pulvinar relationships in these human cases. Four hemispheres with lesions above the level of the splenium of the corpus callosum (medial parietal region) were associated with concomitant cell loss in the superior portion of Pu m. Lesions below the level of the splenium of the corpus callosum (medial temporo-occipital region) were accompanied by cell loss in the inferior portion of Pu m. The connections along the anteroposterior axis of the pulvinaris medialis followed the anteroposterior axis of the medial hemisphere with the posterior pole of the pulvinar being connected to the medial temporo-occipital junction.

The second volume, entitled VARIATIONS OF THE HUMAN DIENCEPHALON, provides a three-plane reconstruction atlas of the cytology of the thalamic nuclei related to the stereotaxic commissural orientation system. In addition, variation studies are available for 25 brains in each of the three planes again with reference to the standard commissural stereotaxic orientation system. This provides the gross relationships of the thalamus, basal ganglia and upper brain stem.

The last section of Volume II is a study in the three stereotaxic planes of the individual thalamic nuclear variations related to the standard commissural stereotaxic reference system. This was prepared from serially sectioned, celloidin embedded brains. Unfortunately, this material showed differential x-y distortion. This was corrected, however, by digitizing the outlines and applying differential x-y corrections by use of a special computer program provided by Mr. Lawrence Mersereau.

These variation studies are both original efforts in this field.

Significance to Bio-medical Research and the Program of the Institute:

The present study provides a firm anatomical basis for studies of the human thalamus and allows evaluation of the effects of therapeutic lesions in the thalamus and basal ganglia. A number of specimens with old stereotaxic lesions have been collected over the years from the Surgical Neurology program and more are available in the Yakovlev collection in Massachusetts. Study of the material will proceed in attempts to find physio-anatomical correlations and understand the effects of thalamotomy. To date thalamotomy is a purely empirical operation heavily burdened by post hoc reasoning.

Proposed Course of Project:

During the coming year, a special effort will be made to make use of the EM facilities present in the Branch. This provides the possibility of combining Golgi technique to identify the cell and EM techniques to identify the synaptic relationships on the cell. This rather laborious technique has been used with considerable success by a few previous investigators. However, much remains to be done. A special interest will be taken in the squirrel monkey since in this animal the thalamus is much like that of man yet the total volume of the brain is small and relatively easy to handle. This basic work will further support the proposed evaluation of therapeutic lesions cited above.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Trauma of the Nervous System. Experimental and Clinical Studies of the Neural Response to Injury.

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: R. M. Mahone, M.S., K. Post, M.D., R. Crowell, M.D., H. Metz, M.S., M. Major, B.S., I. Klatzo, M.D., B. Whang, Ph.D., and S. Roberts, Ph.D.

Cooperating Units: Protection Systems Branch and Design Applications Branch, NSRDC; Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH; Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS; Department of Mechanical Engineering, UCLA

Man Years

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives:

1. To understand the mechanism of mechanical and ischemic trauma of the nervous system; to be able to predict the results of such trauma in man and to establish optimum principles of protection, prevention and management.

2. The development of our understanding of the biomechanics of the nervous system in health and disease seeking to apply principles of statistical mechanics to neural function and its derangements.

Methods Employed:

1. Electroencephalographic and somatic sensory evoked potentials (SER) have been successfully recorded from the alert restrained chimpanzee without any drugs before and after experimental head injury. The recovery cycle of the SER promises to be a most fruitful avenue for further research in the mechanism of traumatic unconsciousness.

2. A study of traumatic amnesia in the rat using one-trial conditioned response technique for memory testing (Jarvik) while comparing the neurologic behavioral effect of such concussions to that seen after electroconvulsive shock (ECS) using television monitoring techniques has been completed. The amnesic and neurologic effects of head injury in this species were found to be dissociated.

3. The experimental measurement of the amplitudes of brain displacement with and without water immobilization of the head on the body in monkeys subjected to steady-state vibrations up to 30 Hz (1" travel) has been completed.

4. A three-compartment theoretical model of the brain tissue, CSF and blood volumes is being subjected to experimental test in the monkey in an attempt to define the role of the brain tissue volume in the mechanics of hydrocephalus. The pressure-volume relationship of the CSF in the ventricular system is being documented.

5. Accident data on human injuries in car crashes wherein neural trauma has occurred are being collected from a variety of sources in order to obtain validation of our scaling hypothesis.

6. Two mathematical models of the head and neck are being developed using lumped parameter (with Dr. S. Roberts) and finite element (with Dr. B. Whang) methods.

7. A new apparatus for subjecting the rhesus monkey head to precisely controlled acceleration in either linear or angular modes is being constructed.

8. A model for ischemic trauma to the monkey brain has been developed and investigated by radiologic and neuropathologic techniques.

9. A model for controlled mechanical trauma to the spinal cord of monkeys is being developed.

Major Findings:

1. Preliminary data analysis on the squirrel monkey and chimpanzee impact and whiplash injury experiments indicate encouraging fit of experimental levels to predicted levels for onset of cerebral concussion in these species in terms of rotational acceleration. Data for head impact tolerance levels (in terms of cerebral concussion onset) however are incomplete, particularly in the chimpanzee. These data do suggest however that structural factors (skull and scalp thickness, brain/head weight ratio) play a much more important role under direct impact conditions. A linear method for scaling the contact phenomena of impact is being developed.

2. The surprising finding of only partial amnesia after concussion in the rat occurred in the presence of severe and long lasting neurologic disturbances (ataxia, decreased mobility) following the impact. These findings are in contrast to electroconvulsive shock which produces complete retrograde amnesia associated with reversible neurologic disturbances of milder degree after the tonic-clonic convulsions cease. A hypothesis implicating the temporal lobes to explain this finding has been developed.

3. A clear demonstration has been obtained for the importance of head rotation in the production of brain distortion and the inability of a

purely linear displacement of the head to produce measurable brain distortions as indicated by radiographic visualization of isodensity pellets in the brain and by cortical displacements seen through a Lexan calvarium with high speed cinematography. Rotational frequency of brain distortion equals 5-10 Hz.

4. Data from the pressure-volume relationship of the ventricular CSF in monkeys suggest that the reduction of brain tissue volume occurs primarily at the expense of the vascular compartment. The crucial factor in the mechanics of hydrocephalus appears to be the rate of increase of unabsorbed CSF.

5. Ischemic trauma (middle cerebral artery occlusion) in the monkey can be tolerated much longer (up to four hours) than hitherto realized.

6. Data testing the lumped parameter mathematical model for head injury have been collected and this model will soon be ready for predictive experimental validation.

7. The studies on injured primate spinal cord have brought out the importance of degree of injury to the vascular structures in the central grey matter as being crucial for the severity of the cord injury.

Significance to Bio-medical Research and the Program of the Institute:

1. The ability to define an injury threshold for cerebral concussion and other effects of nervous system trauma in man suggests the possibility of a more adequate definition of performance criteria for brain protection during head injury and whiplash injury in automobile accidents and motorcycle crashes. This will include the design of helmets of all types as well as automobile interior and exterior design. It is for this reason that our research is being supported by the Department of Transportation.

2. The finding that a cervical collar raises the threshold for experimental cerebral concussion in the monkey suggests a number of ways in which protection against such injury may be achieved under a variety of conditions. Currently an inflatable collar device and an inertia strap for helmet restraint are being tested at the Air Crew Equipment Laboratory in Philadelphia. An application for a patent on an inflatable collar has been submitted.

3. The findings that whiplash injury alone can produce significant degrees of visible brain surface hemorrhages raise the issue of such lesions being produced by whiplash trauma in man. The documentation of this sequelae in two cases supports our scaling hypothesis and is of direct relevance in the design criteria for protective equipment. The consequences of minor degrees of this pathologic effect of indirect impacts for the chronic sequelae of head injuries requires further study.

4. The observations on the alterations of the SER in the chimpanzee and on traumatic amnesia in the rat will be of direct relevance to understanding the mechanism of traumatic unconsciousness.

5. The observations on the mechanics of hydrocephalus can lead to the definition of precise diagnostic-prognostic clinical tests for progressive and static hydrocephalus in humans. Currently a new device for volume regulated treatment of hydrocephalus is under development.

Proposed Course:

1. Input from the ongoing biomechanical, biochemical and physiopathological studies will be used to develop a more sophisticated theoretical analytic model of the behavior of the brain in response to trauma, both static or quasi-static (e.g. hydrocephalus or tumors) and dynamic (e.g. head injuries).

2. Controlled "whiplash" and whole-body acceleration trauma experiments in monkeys will be conducted to round out other aspects of the mechanics of injury in order to complete the definition of *thresholds* of CNS injury. These further tests are based on validation of the theoretical models.

3. Experimental verification of our scaling method $\left(R = \frac{c}{m^{2/3}}\right)$ and further elaboration of scaling methodology using data from impact and whiplash experiments using the squirrel monkey and chimpanzee as well as the rhesus will be completed. Testing of the prediction of concussive threshold for man against available accident data will be pursued.

4. Prophylactic and therapeutic measures aimed at reducing the noxious and enhancing the beneficial responses after trauma will be studied experimentally in the monkey.

5. The neurophysiologic and neuropathologic effects of head injury will be studied in an attempt to define further the mechanism and sequelae of head injury. This will be pursued initially with the effect of trauma on the biogenic amine systems of the brain using the technique of quantitative histochemistry. A visiting scientist, Dr. Nakatani, will be shortly joining us to develop this study.

6. The model for spinal cord injury will be used to investigate recovery and regeneration in primate spinal cord after varying severities of trauma.

Honors and Awards: None

Publications:

Crowell, R.M., Olsson, Y., Klatzo, I., and Ommaya, A.K.: Temporary focal ischemia in the monkey. *Trans. Amer. Neurol. Ass.* (In press).

Crowell, R.M., Olsson, Y., Klatzo, I., and Ommaya, A.K.: Temporary occlusion of the middle cerebral artery in the monkey: Clinical and pathological observations. *Stroke* 1: 439-448, 1970.

Crowell, R.M., Olsson, Y., and Ommaya, A.K.: Angiographic and microangiographic observations in experimental cerebral concussion. *Neurology* (In press).

Hirsch, A.E. and Ommaya, A.K.: Protection from brain injury: The relative significance of translational and rotational motions of the head after impact. In Proceedings of the 14th Stapp Car Crash Conference. New York, Society of Automotive Engineers, Inc., 1970, pp. 144-151.

Hirsch, A.E., Ommaya, A.K., and Mahone, R.M.: The tolerance of sub-human primate brain to cerebral concussion. In Gurdjian, E.S., Lange, W.A., Patrick, L.M., and Thomas, L.M. (Eds.): Impact Injury and Crash Protection. Springfield, Ill., Charles C. Thomas, 1970, pp. 352-371.

Letcher, F.S., Corrao, P.G., and Ommaya, A.K.: Spontaneous and evoked epidural potentials in the head injured chimpanzee. J. Neurosurg. (In press).

Metz, H., McElhaney, J., and Ommaya, A.K.: A comparison of the elasticity of live, dead and fixed brain tissue. J. Biomechanics 3: 453-458, 1970.

Ommaya, A.K.: Nervous system injury and the whole body. J. Trauma 10: 981-990, 1970.

Ommaya, A.K. and Hirsch, A.E.: Tolerances for cerebral concussion from head impact and whiplash in primates. J. Biomechanics 4: 13-21, 1971.

Ommaya, A.K., Chairman; Walker, A.E. and Gurdjian, E.S., Co-Chairman; Brackett, C.E. and Langfitt, T.W., Rapporteur: Trauma Workshop Report: Neural trauma. J. Trauma 10: 1069-1071, 1970.

Serial No. NDS(I)-62 SN/OC 913(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objective: To study the activity of single nerve cells in the cerebral cortex and subcortical nuclei in relation to motor and sensory functions.

Methods Employed: Two micro-electrodes are placed, one in the motor cortex and the other in the thalamus of patients undergoing operative procedures. The other parameters, e.g. EEG, EMG, are also measured in response to motor movement or sensory stimulation.

Major Findings: Some cells in the motor cortex were found to be active in close relation to motor movement, such as Parkinson tremors, voluntary flexors or extension of the opposite extremity. A few showed suggestive evidence that the activity is dependent upon the activity of the thalamic neurons. The latter observation awaits being confirmed by the computer, which it is now in the process of doing.

Significance to Bio-medical Research and Program of the Institute: The program will add to our knowledge of normal and abnormal functions of the motor system in man.

Honors and Awards: Invitation to a Symposium on Neurophysiology in Man to be held in Paris on July 20, 1971.

Publications: None

Serial No. NDS(I)-63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Glial Tumors of the Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: V. T. Oliverio, Ph.D. and V. T. Marchesi, M.D., Ph.D.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI;
Laboratory of Experimental Pathology, NIAMD

Man Years

Total: .8
Professional: .4
Other: .4

Project Description:

Objectives:

1. To develop improved chemotherapeutic control of inoperable gliomas.
2. To isolate tumor specific antigens from human glial tumors.
3. To develop immunotherapeutic methods for prevention and treatment of glial tumors.

Methods Employed:

1. The cerebrospinal fluid reservoir designed by the principal investigator has been connected to a new device called a "tumor cyst prosthesis". This allows repeated sampling from or instillation within the tumor mass.
2. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grade III or IV) are selected. Adequate standard therapy (surgical decompression, radiation) and the extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically.
3. Cerebrospinal fluid reservoirs are inserted to provide portals for either perfusion or infusion chemotherapy.
4. The drug of choice (8-Azaguanine or CCNU) is administered at intervals for 4 to 6 treatments. Effects are gauged clinically and by

repeat of the neuroradiologic tests.

5. Samples of tumor cells and fluid from within the prosthetic cyst are being used for isolation of tumor specific antigens by techniques based on the experiments of Gold et al.

6. Lipid soluble cytotoxic agents such as CCNU are being tested for topical and intrathecal use in the rhesus monkey.

Major Findings:

This project has been dormant for the past year but is now reactivated by the innovations described in Methods 1, 5 and 6 above. Results to date are too preliminary for comment.

Significance to Bio-medical Research and the Program of the Institute:

It is hoped to improve the presently inadequate treatment of glial tumors of the nervous system and hopefully extend clinical cures to patients with glioblastoma multiforme and other malignant gliomas. The development of immunotherapeutic methods could conceivably lead to preventive techniques of brain tumor management.

Proposed Course:

Lipid soluble cytotoxins which can be used topically will be instilled via the tumor cyst prosthesis in combination with parenteral therapy. The isolation of tumor specific antigens will be used to develop definitive immunotherapeutic methods of brain tumor control.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-66 SN/OC 1245(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: EEG Learning Correlates using Scalp and Intracranial Depth Electrodes.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and William Sheriff, M.A.

Other Investigators: M. Buchsbaum, M.D.; J. Van Buren, M.D., Ph.D.; A. Ommaya, F.R.C.S.; J. Bryan, M.A. and M. Haddox, R.N.

Cooperating Units: Laboratory of Psychology, NIMH; Section on Technical Development, NIMH.

Man Years:

Total:	.3
Professional:	.2
Other:	.1

Project Description:

Objectives: To study the locus and functions of cerebral mechanisms which regulate perception, information storage and retrieval in man.

Methods Employed: Two classes of stimuli, verbal (3-letter words) and nonverbal (nonsense dot arrays) were projected successively in the left or right visual field, approximately 2°21' from a central fixation point. The stimuli were delivered at rapid, tachistoscopic speed and required binocular viewing. Average evoked cortical responses to the discriminanda were calculated simultaneously from left and right occipital EEG tracings in a group of normal subjects and a group of 11 neurosurgical epileptic patients with left or right temporal lobe resections. The LINC computer was used to generate the perceptual stimuli. Digital conversion of bipolar EEG activity and the computation of the evoked responses were performed by the LINC; the entire test session and signal averaging were conducted on-line.

Major Findings: Perceptual discrimination of meaningful and nonsense material was accompanied by dissimilar evoked potentials, the differences being greater for activity recorded from the left hemisphere, regardless of a left or right field presentation. Right hemisphere activity failed to show corresponding differences in evoked potentials, although the two sets of stimuli did evoke different averaged responses when the material was flashed in the left visual field.

This asymmetry in electrographic activity suggests that the exchange of information between the left and right brain in man, via commissures and/or other subcortical pathways, may favor reception by the left hemisphere. The dominant language brain (usually left) therefore appears to have access to a readout of information delivered to or processed by the right hemisphere, and an inherent capability to assume a comparator role in certain stages of perception. The results also indicate that the dominant nonverbal brain (usually right), or those cerebral mechanisms which are more intimately concerned with visuomotor, spatial abilities, participate to some degree in language functions.

In accord with clinical observations, the expected verbal impairment for left temporal lobotomy cases was expressed in undifferentiated averaged waveforms during presentation of verbal material. The predicted nonverbal deficit for the right temporal lobotomy patients was represented by poorly defined potentials recorded during perception of the nonsense dot patterns. The right temporal group also exhibited poorer averaged EEG responses from both hemispheres during visual discrimination, suggesting a more pervasive perceptual impairment.

Significance to Bio-medical Research and the Program of the Institute: Behavorial data available from patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits, dependent upon the verbal or nonverbal nature of the material. The technique employed in this project affords a more precise method for examining participation by cortical and subcortical structures during the course of perception and learning and modification of human behavior.

Proposed Course of Project: To develop additional tasks and programs for EEG data analysis with wider application to patients with different neurologic disorders.

Honors and Awards: None

Publications:

Fedio, P. and Buchsbaum, M.: Unilateral Temporal Lobectomy and Changes in Evoked Responses during Recognition of Verbal and Nonverbal Material in the Left and Right Visual Fields, Neuropsychologia, (in Press).

Serial No. NDS(I)-67 SN/OC 1417(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Joseph Fenstermacher, Ph.D., Barbro Johansson, M.D.

Cooperating Units: C-LCP

Man Years:
Total: 0.4
Professional: 0.3
Other: 0.1

Project Description:

Objective: To determine the resistivity of nervous tissues under various neurosurgical conditions.

Methods Employed: Cats under Fluothane anesthesia, hypothermia, hypertension, etc. were used. In one experiment, a monkey was used who was subjected to electrical measurements for a period of two months. The method of measuring electrical resistance of the tissue was previously described (Li, et al. 1968). With this method, the results were applied to the Maxwell's Equation and the extracellular space of the tissue was calculated. The calculated extracellular space was then compared with the findings obtained by the concentration profiles of radioactively labeled compounds following subarachnoid perfusion. The brains of the animal after the experiment were fixed, sectioned and stained with Luxol Fast Blue-Nissl and Luxol Fast Blue-PAS stains for histological examination.

Major Findings: The extracellular space of the cat's cerebral cortex was found to be between 15 and 20% and was unchanged when the temperature of the cortex was decreased from 37 to 15° C. The rate of CSF formation was, however, found to be markedly reduced by hypothermia.

In another series of experiments, a sudden increase of systemic blood pressure by 90 mmHg was found to break down the blood brain barrier.

With the blood brain barrier broken down, the activity of the nerve cells was found to be changed or inhibited.

The results from experiments on brain edema show changes in the level of consciousness which are still being subjected to analysis.

Significance to Bio-medical Research and Program of the Institute: Change in extracellular space may be a measure of extracellular or intracellular edema of the cells. The latter, in turn, is a measure of the metabolic response of the nerve cells to various pathological or physiological conditions. It is hoped that the method herewith described will be applied to neurosurgical patients as a measure of the progress or improvement of the neurological conditions.

Proposed Course of Project: Further study will be made in chronic monkeys. The experience with monkeys will lead to the development of a better method which can be applied to clinical patients.

Honors and Awards: None

Publications:

Fenstermacher, J. D., Li, C-L. and Levin, V. A.: Extracellular Space of the Cerebral Cortex of Normothermic and Hypothermic Cats. Exp. Neurol., 27: 101-114, 1970.

Johansson, B., Li, C-L., Olsson, Y. and Klatzo, I.: The Effect of Acute Arterial Hypertension on the Blood-Brain Barrier to Protein Tracers. Acta Neuropath, 16: 117-124, 1970.

Serial No. NDS(I)-67 SN/OC 1418(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Surgery of the Spinal Cord

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: G. Di Chiro, M.D.

Cooperating Units: None

Man Years

Total:	.6
Professional:	.3
Other:	.3

Project Description:

Objectives:

1. To determine the etiology of non-traumatic myelopathies and particularly to establish the pathological anatomy and natural history of spinal arteriovenous malformations.

2. To develop new and improved techniques for surgical treatment of spinal cord diseases.

Methods Employed:

1. Selective angiography of the spinal cord is used to display the vascular morphology of lesions in this area.

2. Microneurosurgical techniques are being developed in the course of surgical treatment of patients as well as in sub-human primates.

Major Findings:

Sixty-five patients with spinal cord arteriovenous malformations and ten patients with tumors of the spinal cord have been studied to date.

1. Our initial classification of spinal cord arteriovenous malformations (based on the angiographic anatomy) into three types, Type I - single coiled vessel, Type II - glomus, and Type III - juvenile, is holding up under scrutiny of our increasing experience. Our hypothesis that the

surgical treatment required varies with the type of lesion is also supported. Thus the Type I is amenable to embolization, arterial ligation or excision, Type II is best treated by excision and Type III by embolization or ligation.

2. Selective embolization of the anterior spinal artery with autopolymerizing silicone rubber as a technique for developing an experimental model for spinal cord infarction in the monkey is being investigated.

3. Microsurgical techniques specific to spinal cord lesions have been developed and are being improved.

Significance to Bio-medical Research and the Program of the Institute:

In addition to providing improved management of spinal cord disease our surgical methods and diagnostic studies provide an opportunity to contribute significantly to the knowledge of the normal and abnormal blood supply of the human spinal cord as well as to related aspects of spinal cord physiology and pathology.

Proposed Course:

1. To investigate the epidemiology and etiology of non-traumatic myelopathies in collaboration with Professor Chatterjee of Calcutta, India, via the mechanism of an NINDS supported PL 480 funded project.
2. To develop definitive standards for spinal cord surgery for all types of lesions.
3. To study the mechanism of ischemic injury to the spinal cord.

Honors and Awards: None

Publications:

Doppman, J.L., Di Chiro, G., and Ommaya, A.K.: Percutaneous embolization of spinal cord arteriovenous malformations. J. Neurosurg. 34: 48-55, 1971.

Kaufman, H.H., Ommaya, A.K., Doppman, J.L., and Roth, J.A.: Cord syndrome secondary to hypertrophy of the ligamentum flavum in an acromegalic. Arch. Neurol. (In press).

Ommaya, A.K.: Arteriovenous malformations of the spinal cord. In Youmans, J. (Ed.): Neurological Surgery. Philadelphia, Pa., W.B. Saunders Company, 1971. (In press).

Serial No. NDS(I)-67 SN/OC 1424(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and Ayub K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., Ph.D.; T. Zahn, Ph.D.; W. Sheriff, M.A. and M. Haddox, R.N.

Cooperating Units: Laboratory of Psychology, NIMH and Section on Technical Development, NIMH

Man Years:

Total:	.3
Professional	.2
Other:	.1

Project Description:

Objectives: To identify structural-functional interplay of brain mechanisms which regulate intellectual and emotional behavior in man.

Methods Employed: Therapeutic benefits from bilateral cingulumotomy in reducing 'appreciation of pain' are examined by standard psychophysiologic and behavioral measures prior to and following surgery. Performance on a variety of perceptual and short-term memory tasks is also studied during electrical stimulation of chronic electrodes implanted in the cingulum or temporal lobe structures of the limbic system.

Major Findings: Five patients undergoing bilateral cingulumotomy for the relief of pain were examined on standard clinical tests of intelligence and memory (Wechsler Forms). Prior to therapeutic coagulation, patient performance was also monitored during electrical stimulation with electrodes chronically implanted in the left and right cingulum.

A severe impairment in recall for verbal memoranda followed left, and not right cingulum stimulation. Further inspection of the memory disorder showed that, while sparing perceptual processes, cingulum stimulation may have upset the control mechanisms responsible for retrieving information from short-term memory storage.

The deficit associated with cingulum stimulation is dissimilar to that observed with left thalamic stimulation where transient language difficulties resulted from stimulation within the pulvinar nucleus [Project 200(c)].

Psychometric observations after bilateral cingulumotomy failed to show comparable deficits in memory and intellectual achievement. This apparent dissociation in the presence of clinical improvement adds support to the role of the cingulum as a modulator of emotional responsiveness, implicating more distal limbic mechanisms for memory functions.

Significance to Bio-medical Research and the Program of the Institute: This project provides an opportunity to define precise behavioral and autonomic concomitants of limbic functions in man. The observations contribute to a better understanding of psychophysiologic mechanisms related to modifications in the apperception of pain and stress following cingulumotomy.

Proposed Course of Project: To continue the study and to extend the protocol to include patients subject to therapeutic lesions in the temporal lobe.

Honors and Awards: None

Publications:

Fedio, P. and Ommaya, A. K.: Bilateral Cingulum Lesions and Stimulation in Man with Lateralized Impairment in Short-Term Verbal Memory, Exp. Neurol., 29: 84-91, 1970.

Serial No. NDS(I)-68 SN/OC 1522(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1970

Project Title: Neural Mechanisms for Learning and Memory

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: P. Fedio, Ph.D., J. Bossom, Ph.D., and
D. Levine, M.D.

Cooperating Units: Primate Laboratory, Queens College, New York;
Laboratory of Neural Control, NINDS

Man Years

Total: .6
Professional: .3
Other: .3

Project Description:

Objectives:

1. To determine the effect of sensory deafferentation on learning.

2. To determine the mutual roles of complexity and time in the mechanism of memory and develop a predictive theory of memory.

3. To ascertain the role of the limbic system in memory and visuo-motor learning.

Methods Employed:

1. Prism adaptation in monkeys combined with classical lesion analysis using behavioral techniques is the main technique. A test for reaching-grasping behavior has been developed by Dr. Joseph Bossom.

2. A matching from sample memory test capable of controlled variation of time and complexity of task has been completed.

3. Selected patients admitted for surgical relief of either pain or epilepsy often need depth electrodes implanted for therapeutic purposes in either the cingulum or hippocampus-amygdala respectively. Psychologic and memory tests before, during and after stimulation and coagulative electrode lesions at these sites provide important data for analysis.

Stimulation of these structures at various parameters of current and frequency is carried out before the lesion is made.

Major Findings:

1. Left cingulum stimulation impairs verbal short-term memory while non-verbal memory is impaired by right cingulum stimulation. Equivalent stimulation of the hippocampus and amygdala (right or left) appears to be less effective in disrupting such tasks. A new hypothesis for the role of limbic structures in memory has been proposed.
2. Unilateral temporal lobectomy in man uniformly produces memory defects related to disability in learning verbal memoranda (after left lobectomy) and non-verbal memoranda (after right lobectomy).
3. After bilateral dorsal rhizotomy, monkeys can learn to discriminate small differences in force of hand closure.
4. After unilateral dorsal rhizotomy in monkeys simple restraint of the normal arm allows use of the deafferented arm for reaching and grasping and a hitherto apparently paralyzed arm can be made useful.
5. Bilateral dorsal rhizotomy in monkeys (C2 to T4) does not obliterate reaching-grasping and prism adaptation is unimpaired. This finding supported older theories of a "motor correlary discharge" capable of providing sufficient "local feed-back" in the brain to perform useful motor acts. Lesions in the temporal, striate and parietal cortex do not impair this mechanism which is inhibited only by frontal lobe lesions (involving head of caudate nucleus or bilateral stereotaxic caudate nucleus lesions).
6. Aspects of the work on cingulum stimulation have been reported in publications listed under Project Serial No. NDS(I)-67 SN/OC 1424(c).

Significance to Bio-medical Research and the Program of the Institute:

It is hoped that the understanding of visuo-motor learning and memory will lead to a more accurate system of guiding the acquisition of neonatal and infant motor skills as well as improve the rationale and quality of rehabilitation after severe neurologic disease with crippling sequelae. The understanding of memory mechanisms linked to motor expression of learning suggests useful application to improved educational and rehabilitative methods.

Proposed Course:

Methods of accelerating relearning of motor function after sensory deafferentation will be developed in sub-human primates. Our hypothesis for the role of limbic structures in memory and learning will be tested and developed in man. Further studies in this aspect will include the search for quantitative electrophysiologic correlates of memory and learning.

Honors and Awards: None

Publications:

Bossom, J. and Ommaya, A.K.: Voluntary movement in deafferented monkeys: Characteristic ataxia and the effect of caudate nucleus lesions. Arch. Neurol. (In press).

Ommaya, A.K. and Fedio, P.: The contribution of cingulum and hippocampal structures to memory mechanisms. A study with chronic electrodes in man. Proc. Amer. Assoc. Neurol. Surgns. April 1971. (In press).

Serial No. NDS(I)-68 SN/OC 1526(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Dr. Vazha Mikhailovich Okujava, Mr. Anthony Frank Bak and Dr. Claire Parsons

Cooperating Unit: Laboratory of Neurophysiology, NIMH

Man Years:

Total:	0.2
Professional	0.2
Other:	0

Project Description:

Objective: To study the electrical properties and the responses to electrical and pharmaceutical stimulation of the normal and epileptic nerve cells in the cerebral cortex by means of intracellular microelectrodes.

Methods Employed: Adult cats were used. They were initially anesthetized with intraperitoneal pentobarbital sodium. The trachea was cannulated for artificial respiration and the anesthesia was supplemented with d-tubocurarine. A glass micropipette electrode was inserted into a cell in the cerebral cortex and measurement was made simultaneously with a gross electrode placed on the surface of the cortex. Electrical stimulation was applied either through the micropipette recording electrode or through the surface electrode. The results are to be compared with those in experiments in which convulsive chemical agents had been applied to the surface of the cortex.

Major Findings: The analysis of the results of the first series of experiments on normal cortical neurons has been completed. Electrical properties of the cerebral cortical elements were examined by passing current through intracellular micropipette electrodes. It was found that the threshold strength for direct excitation of neurons was 0.20 - 0.40 x 10^{-9} amp. Upon supramaximum strength of stimulation, the neurons discharged at high frequencies up to 500 per sec; the frequency of discharges rapidly reduced and in 40 - 110 msec became fairly constant at 45 - 200 per sec. The response to direct excitation was followed by hyperpolarization; however, if the excitation was immediately preceded by a prolonged passage of negative current, hyperpolarization tended to be replaced by depolarization and spike discharges. Further, during the period of hyperpolarization the input resistance of the neuron was markedly reduced. Resting membrane potentials of cortical neurons were found to be 59.92 ± 7.45 (SD) mV; of the inexcitable elements, presumably glial cells, 70.08 ± 8.62 (SD) mV. Of the neurons, the resistance was 9.17 ± 2.68 (SD) x 10^6 ohms; capacitance, 0.69 ± 0.35 (SD) x 10^{-9} farads; and time constant, 5.69 ± 1.34 (SD) x 10^{-3} sec. Of the inexcitable elements, the resistance was in the order of 2.40 x 10^6 ohms; capacitance, 1.36 x 10^{-9} farads; and time constant, 2.83 x 10^{-3} sec.

The results of the second series of experiments are subjected to detailed analysis at present, and conclusions will be presented in the near future.

Significance to Bio-medical Research and Program of the Institute: The findings which reveal the basic electrical properties of the cells in the cerebral cortex strongly indicate that they are different from cells under pathological or abnormal conditions.

Proposed Course of Project: Measurements will be made on nerve cells in edematous cortex and those in animals under hyper- and hypotension. Similar measurements will also be obtained in cells of different types of brain tumors. It is believed that these measurements may be used as an indication of the success or failure of different kinds of chemotherapy in brain tumors.

Honors and Awards: None

Publications:

Li, C-L., Okujava, V. M. and Bak, A. F.: Some Electrical Measurements of Cortical Elements and Neuronal Responses to Direct Stimulation with Particular Reference to Input Resistance. J. Exp. Neurol. (in press).

Serial No. NDS(I)-68 SN/OC 1527(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: David Bachman and Levon Parker

Cooperating Unit: None

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathways.

Methods Employed: Intracellular and extracellular micropipette electrodes were used to record the activity of the nerve cells in the motor cortex and stimulation was applied to the basal nuclear structures.

Major Findings: In one series of experiments it was established that the activity of the cells in the red nucleus could be increased or decreased by stimulation of the contralateral or ipsilateral cerebellar fastigial nucleus. A conclusion was also arrived at that these are polysynaptic pathways from both fastigial nuclei to the red nucleus.

The results of two other series of experiments have been analyzed and are ready for publication.

Significance to Bio-medical Research and Program of the Institute: This study provides further knowledge of the function of the pyramidal and extrapyramidal systems which have been known to be directly involved in motor activity.

Proposed Course of Project: In the future when all the pathways and their interaction are further understood, experiments will be designed to study the mechanism of motor dysfunction by electrical stimulation and chemo-injection in various basal nuclei. Experiments will also be carried out on monkeys with lesions in the basal nuclei, and neurophysiological and neurological recordings will be made in the monkey with and without electrical stimulation or chemo-injection.

Honors and Awards: None

Publications:

Bachman, D. and Li, C-L.: The Effect of fastigial Stimulation on Neuronal Activity in the Red Nucleus. J. Exp. Neurol. (in press).

Ratcheson, R. A., Wirth, F. P., Jr., Li, C-L. and Van Buren, J. M.: Syringobulbia - Prolonged Survival. Acta Neurochir. (in press).

Serial No. NDS(I)-68 SN/OC 1658(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hemispheric Development and Specialization of Intellectual Functions.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and John Van Buren, M.D., Ph.D.

Other Investigators: A. Ommaya, F.R.C.S.; S. Goodman, Ph.D. and R. Minsky, Ed.D.

Cooperating Units: Division of Research and Psychological Services, Montgomery County Schools, Maryland

Man Years:

Total:	.8
Professional:	.6
Other:	.2

Project Description:

Objectives:

1. Compare the effects of brain lesions sustained during infancy or adulthood on the development and recovery of intellectual abilities.

2. Outline neural code for perceptual and memory skills which support language and spatial judgment.

3. Examine developmental competence of brain mechanisms involved in reading and related functions.

Methods Employed:

1. With neurosurgical patients being considered for unilateral temporal lobectomy, lateralization of speech to the left or right hemisphere is determined by the intracarotid Amytal (WADA) procedure. Verbal behavior (name-recall on a serial task) was continually monitored before and after left and right intracarotid injections of Amytal to provide observations on baseline, dysphasia and recovery performance.

2. The laterality and general outline of cerebral zones instrumental in perception and immediate memory of verbal and nonverbal material were mapped by cortical stimulation during neurosurgical treatment of epileptic patients. Behavioral observations were monitored on a serial, object-naming task and a random form discrimination test, each with a short-term memory command.

3. Children with reading disorders are presented with perceptual tasks which require recognition for verbal material projected at tachistoscopic speed to various points in the visual field.

Major Findings:

1. Amytal irrigation of the hemisphere dominant for speech produced marked slowing in the recovery of basic linguistic skills and a longer-lasting deficit in immediate memory for verbal material. Conversely, intracarotid injection of Sodium Amytal on the contralateral, nondominant side yielded no anomia and had considerably less effect upon recall before pre-injection or baseline verbal performance was recovered.

Following injection of Amytal, particularly in the left, the uneven recovery of language reflected a differential threshold to drug action within the hierarchy of cerebral organization of verbal processes. At an elementary level, neural mechanisms involved in motor production of basic speech patterns appeared to be spared from the influence of Amytal, regardless of left or right injection. At an intermediate level, the recovery of speech and test orientation did not preclude the emergence of difficulty in naming simple objects.

Finally, the drug related difficulties in response integration were most exaggerated in memory spheres. After the dysphasic condition was totally remitted, the patients showed prolonged impairment in short-term verbal memory, illustrated by an inability to hold and to recall the name of any object that had been correctly identified 12 - 15 seconds earlier.

2. Reversible dysphasia was induced by brief electrical stimulation of sites within the posterior temporo-parietal cortex (Wernicke's Area) in the hemisphere that has major control over verbal behavior. In the absence of dysphasia or perceptual faults, stimulation in the language zone also produced a marked retrograde loss in rote memorization of a single object name. Excitation of the anterior, lateral temporal neocortex, in contrast, did not interfere with object identification and, instead, resulted in an anterograde defect in immediate memory. This preliminary evidence tentatively suggests that short-term or buffer memory is served by the temporal lobe; long-term storage appears to be more intimately linked with the posterior temporal-parietal system which is least indispensable for receptive and expressive language.

3. Sixth grade children, classified according to poor, average or superior reading grade-scores, achieved comparable recognition thresholds for single alphabet letters presented in the left, central and right visual fields.

Each group required longer tachistoscopic exposure intervals to identify 3-letter words, the magnitude of the thresholds being clearly ranked with the lowest threshold for the superior readers, intermediate for the average reading group and the highest recognition threshold being obtained by children with reading disability.

The left-right field differences for the average and superior reading cases were in accord with the hypothesis that verbal material flashed in the right field is more efficiently handled via direct access to the left dominant-verbal brain than material which appears in the left visual field. In contrast, the poorer readers showed smaller differences in thresholds across fields, and for several students, a reversed, left field superiority was recorded. These findings suggest that reading skills depend upon highly organized neural processes, and that certain forms of reading disabilities may result from inadequate or incomplete development of hemispheral control of language.

It was also interesting to note that within each reading group, girls had lower (better) recognition thresholds than boys. This is consistent with developmental evidence that girls generally exhibit and maintain earlier and superior verbal skills over boys. Apart from the argument that a cultural bias may favor a verbal advantage for girls, the present findings suggest that girls may command a more competent neurolinguistic system than boys.

Significance to Bio-medical Research and the Program of the Institute: The investigations advance understanding of the development and organization of structural-functional relationships in the human central nervous system. The information also prompts attention to the impact of minimal cerebral dysfunctioning in producing dysphasia, dyslexia and kindred language disorders.

Proposed Course of Project: The assortment of behavioral tasks employed will be modified and presented to adult patients with lateralized cortical and subcortical lesions. The study of interhemispheric relations in perceptual style will be continued with dyslexic children with emphasis on more varied, complex visual and auditory stimuli. The possible application for early detection and subsequent remediation of reading problems is also being drafted.

Honors and Awards: None

Publications:

Fedio, P. and Weinberg, L.K.: Dysnomia and Impairment of Verbal Memory Following Intracarotid Injection of Sodium Amytal, Brain Res. (in Press).

Serial No. NDS(I)-60 SN/CN 706(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical, genetic and biochemical studies of progressive cerebral degenerations, mental retardation and cerebral palsy.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Kenton Holden, M.D.

Cooperating Units: Pediatric Metabolism Branch, NIAMD

Man Years:

Total:	1.5
Professional:	1.2
Other:	0.3

Project Description:

Objectives: Chronic neurological disorders affecting children constitute one of the greater medical and social problems of our times. According to conservative estimates for this country, over four million people are permanently handicapped by mental retardation, cerebral palsy, epilepsy or progressive cerebral degeneration. Many of them have to be cared for to the end of their life by the joined services of the family, society and government. Until recently, these conditions did not receive sufficient attention in the field of medical research. Our main objectives are:

1. To subdivide the patients with mental retardation, cerebral palsy and epilepsy into distinct clinical categories using developmental and neurological examinations along with special laboratory procedures.

2. To establish types of cerebral lesion characteristics of each category whenever possible.

3. To apply basic biochemical and genetic methods to selected diseases and syndromes within each category with the aim of advancing the underlying etiology and pathogenesis.

4. To actively seek and advance the novel therapy and prevention of these chronic diseases.

Methods Employed:

1. Detailed general medical and neurological studies.

2. Developmental and psychological examinations.

3. Modified electroencephalographic studies.

4. Genetic and cytogenetic studies.

5. Employment of special procedures such as pneumoencephalography and angiography as indicated.

6. Application of basic biochemical methods to live brain tissue obtained during diagnostic biopsies. Specifically: (a) determination of lipid in the cerebral cortex and white matter, (b) determination of content and individual brain proteins, (c) determination of total and individual polysaccharides and (d) determination of specific enzyme activities.

7. Establishment of the tissue cultures from patients with metabolic disorders and their utilization for biochemical studies.

8. Biochemical assays in the blood, CSF and urine of specific proteins, lipid and carbohydrate metabolism.

Patient material: 24 inpatients (a total of 920 patient days) and 22 outpatients were studied under this project.

Clinical Project:

Major Findings:

1. Patients with newly delineated genetic disease named G_{M1}-gangliosidosis and also those suffering from juvenile type of cerebral lipidosis were subjects of special studies. The investigations were highlighted by the application of novel enzyme procedures and complete determination of all partitioned lipids and glycolipids in the cerebral cortex and white matter obtained by biopsy for diagnostic purposes. These studies were correlated with the result of pathological, histochemical and clinical investigations. Table 1 (on next page) lists distribution of brain lipids in G_{M1}-gangliosidosis and in the juvenile cerebral lipidosis in mg/100 mg dry weight. Certain of these determinations were the first ever to be performed (lipid galactose, glucose, and galactosamine; complete partition of phospholipids).

Table 2 shows clear deficiency in β-galactosidase in G_{M1}-gangliosidosis while β-glucosamine and β-N-acetylgalactosaminidase are normal. Significantly the test also reveals the heterozygous parent; the finding, which if further confirmed, can be applicable to future marital counseling.

TABLE 1

DISTRIBUTION OF BRAIN LIPIDS IN G_{M1}-GANGLIOSIDOSIS AND IN THE JUVENILE CEREBRAL LIPIDOSIS IN mg/100 mg DRY WEIGHT

	GREY MATTER			WHITE MATTER	
	R.F. (G_{M1})	T.P. (JAI)	Normal	T.P. (JAI)	Normal
Water	-	82.85	80.4	74.11	73.9
Defatted dry wt.	43.41	53.52	-	25.35	-
Lower phase lipid	43.41	38.82	41.0	62.32	54.6
Proteolipid protein	3.90	4.45	-	10.24	-
Cholesterol	9.4	6.6	9.6	12.15	15.7
Phospholipid	22.4	25.2	29.4	24.7	28.5
Phosphatidyl ethanolamine	4.9	5.0	8.2	7.39	9.7
Lecithin	9.6	10.0	11.0	7.32	8.4
Sphingomyelin	4.2	4.5	5.0	4.86	5.1
Phosphatidyl serine + inositol	3.7	5.7	5.2	5.18	5.4
Lipid galactose	2.16	0.89	0.66	2.97	2.68
Lipid glucose	.92	.049	.037	.065	.016
Lipid galactosamine	.48	<.01	<.01	<.01	<.01
Sialic acid	2.20	.574	.340	.146	.038

TABLE 2

	β-glucosidase	β-galactosidase	β-N-acetyl galactosaminidase
Case 2 (M.F.)	4.4	4.0	60.9
Father	3.8	55.3	66.8
Normal Range (28)		80 - 160	

It is of considerable importance that our patients with G_{M1}-gangliosidosis did not have hepatosplenomegaly but they did have nests of abnormal "foam" cells in the parenchyma of the liver and spleen. The main histological lesion of the brain of these patients consisted of depletion of cortical neurons with distension of those remaining with a substance which stained positive with PAS and lipid reagents. Biochemical study of the cerebral cortex obtained at biopsy showed seven-fold increase of G_{M1}-ganglioside and its asialo derivative and a reduced amount of phospholipid. White blood cell β-galactosidase activity in the patient was less than 5% of normal and the value for the parent examined was consistent with the heterozygous state (less than 50% of normal).

There is considerable controversy raging about the presence of abnormality in the metabolism of mucopolysaccharides in G_{M1}-gangliosidosis. Wolfe _et al_ (Neurology _20_: 23, 1970) found increased urinary excretion of AMPS in his patient. Our very complete studies did not confirm this. Urinary acid mucopolysaccharides (AMPS) in our patients were investigated in considerable detail on several 24-hour urine collections. Both the CPC precipitation and Ecteola column procedures were used to isolate AMPS. The total amount (average) of urinary AMPS in this patient was 4.0 mg/24 hours expressed as hexuronic acid (Carbazole) which is well within our normal limits of 2.5 - 6.0 mg. The hexosamine values for the CPC precipitated AMPS and the ratios of uronic acid to hexosamine and of glucosamine to galactosamine were as follows:

	Hexosamine mg/24 hours	Uronic Acid / hexosamine	Glucosamine / Galactosamine
Case 2 (M.F.)	3.2	1:1.3	1:1.3
Control	7.3	1:1.03	1:1.2

Clearly there is no significant difference in these values between the patient and the normal control; also the degree of metachromasia of the isolated AMPS was comparable to that of the controls. These last findings make the presence of a significant amount of highly sulfated keratosulfate unlikely.

The patient with juvenile cerebral lipidosis also had an excess of lipid staining material in the grey matter although the distension of the neurons was less marked than that found in G_{M1}-gangliosidosis. The values of grey and white matter cholesterol and phospholipid were subnormal and there was a moderate increase in the total gangliosides in the white but not in the grey matter; the distribution of gangliosides was normal in both grey and white matter. The marked differences in clinical manifestation of these two categories of patients may depend as much on the type of material stored as on the total amount.

In addition to bringing new information these investigations indicate the need for further multidisciplinary studies of brains and visceral organs in all types of patients with cerebral lipid storage diseases. Only when this has been done can a reasonable working classification of these disorders be made.

2. A number of investigators reported increase in acid mucopolysaccharides in fibroblasts tissue culture of the patients with cystic fibrosis (Danes and Bearn, Biochem. Biophys. Res. Comm. 36: 919, 1969; Matalon and Dorfman, Biochem. Biophys. Res. Comm. 33: 954, 1968). Since our technique of determination and analysis of AMPS is considered uniquely sensitive and reliable we have undertaken a detailed study of urinary AMPS in seventeen patients with cystic fibrosis in collaboration with the investigators from NIAMD. The complete data are too voluminous to show here in a table. The overall findings can be summarized as follows: the total urinary AMPS in 8 out of 17 patients with cystic fibrosis were slightly although not significantly elevated and in the remaining 9 they were within the range of normal controls + 2 S.D. In five patients, who were in relapse and receiving Gentamycin, the urinary AMPS exhibited abnormal patterns on paper electrophoresis showing presence of 40-50% heparitin sulfate-like material with the molecular weight of about 25,000. In all these five patients the abnormality reverted to normal when they were improved and off Gentamycin. The possible reasons for these findings are discussed; when various extraneous factors are involved special caution is advised to avoid dangers in misinterpretation and generalization. The distribution of molecular weights in the isolated AMPS was normal in 15 patients; in the remaining two patients there was a consistent marked shift toward the small molecular weight (Mw about 5,000) in comparison to normal controls (Mw about 11,000). These two patients had also slightly elevated total urinary AMPS and could represent a separate variant of the disease.

3. Therapeutic approaches to modify the course of the disease in Hunter-Hurler's patients. Of the following compounds clinically investigated: Vitamin A, salycylates, thyroid, Aralen, glucocorticosteroids, the last compounds provide some promise when tried as a long term therapy using alternating-day dosage. We need to accumulate more patients treated with this regimen before quantitative and statistical evaluation can be made. To date we have four such patients on long-term therapy.

Significance to Bio-Medical Research and the Program of the Institute: The described examples of the findings constitute significant contribution to the research on genetic, metabolic and chronic neurological disorders in children.

Proposed Course of the Project: This field of study is one of the most important in medicine at the present time; therefore, this program should continue for many years to come.

Honors and Awards:

1. Clinical Associate Professor of Neurology, George Washington University (no remuneration).

2. Consultant, Children's Hospital of the District of Columbia (no remuneration).

Publications:

1. Patton, V.M. and Dekaban, A.S.: G_{M1}-gangliosidosis and juvenile cerebral lipidosis. Clinical, histochemical and chemical study. Arch. Neurol., in press, 1971.

2. Constantopoulos, G., Dekaban, A.S., Lapey, A., and di Sant'Agnese, P.A.: Urinary mucopolysaccharides in cystic fibrosis. J. Pediat., in press, 1971.

Serial No. NDS(I)-60 SN/CN 707(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pathological and Biochemical Abnormalities in Brain Occurring During Prenatal and Postnatal Life. Correlations With Mental and Motor Development.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Dennis F. Cain, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: In the majority of patients the causation and the underlying pathology of mental deficiency and cerebral palsy are poorly understood. Detailed histological, histochemical and biochemical examinations of the brains of such children are urgently needed. The main purpose is to correlate the encountered lesions with the clinical data. The accumulation of a larger body of data is expected to provide valuable information regarding pathogenesis and also clues for certain etiological factors.

Many of our patients with progressive cerebral degenerations have either excessive storage of a material in the nervous system (lipid, mucopolysaccharide, glycogen) or loss of structural material (diffuse sclerosis, subacute sclerosing encephalitis, axonal degeneration). Experimental animals will be used to establish biochemical techniques for analysis of partitioned lipids and mucopolysaccharides in subcellular fractions. Human material, both normal and abnormal, will be utilized principally as it becomes available from brain biopsies and at autopsy.

Methods Employed:

1. Completion of comprehensive developmental and neurological protocols of the studied inpatients and outpatients.

2. Performance of routine and special laboratory procedures including specific enzyme assays on plasma and white cells, pneumoencephalography,

angiography, brain scanning and brain biopsy when indicated for diagnostic purposes.

3. Utilization of fibroblast and other types of tissue culture from the patients suffering from various metabolic diseases affecting the nervous system for enzyme studies and for models in therapeutic trials.

4. Use of experimental animals to develop and validate semiquantitative methods for assessing structural and biochemical parameters of brain maturation in normal and health compromised clinical conditions.

5. Utilization of brain biopsies as well as normal and pathological cerebral tissue from autopsies of patients of various ages. The tissues will be subjected to histological, histochemical and biochemical studies.

Major Findings:
1. A comprehensive study of congenital malformations affecting the nervous system was conducted. The novel approach consisted of correlating the following aspects:

(a) Normal development of the nervous system in man during prenatal life.

(b) Morphological analysis of the pathological conditions which affected development of different ontogenetic structures.

(c) Determination of the teratogenetic period when the malformation had occurred.

(d) Analysis of the wide spectrum of clinical features in specific types of brain malformation.

(e) Defining of likely etiological factors involved from the analysis of a-d; to be used for future epidemiological and experimental studies.

(f) Suggestion of treatment and long range management.

Because of the large scope and complexity of the subject studied only certain representative findings will be listed here. The extensively illustrated treatise is in press under the title: "Malformations of the Nervous System. Medical Aspects." (see below). Selected findings and conclusions:

(a) The overall incidence of congenital malformations of the nervous system is about 79% of all types of malformations including multiple anomalies.

(b) Using our own material of human embryos, an outline of the early development of the nervous system was provided. Thus, at about 22 days of gestation (d.g.), the neural folds in the cephalic region are flaring widely. At about 23 d.g. the neural folds begin to close at the level just posterior to the hindbrain. By about 24 d.g. the neural tube is closed as far rostrally as the midbrain and the primordium of the main subdivisions of the brain, namely: forebrain, diencephalon, midbrain and hindbrain are indicated by specific constrictions and growth enlargement. About 28 d.g. the neural tube is closed but the brain still consists of a single cavity uniform in thickness. The first evidence of formation of the two cerebral hemispheres is seen at about 31 d.g. By 52 d.g. the main subdivision of the diencephalon can be made out and the cerebral pallium consists of three separate layers: germinal zone, mantle layer and a thin cortical zone. In fetuses of five months of gestation the migration of the neuroblasts to form cerebral cortex is completed but the individual layers are not yet clearly formed. From this time onwards there is a quite rapid progress in the formation of convolutions and sulci as well as final stratification of the cortex; these changes being largely completed by seven months of gestation.

(c) From the knowledge of these developmental landmarks the following conclusions on the teratogenetic periods can be drawn: dysraphic states involving the spinal cord and brain must occur prior to the complete closure of the neural tube, that is prior to 28-29 d.g. Arhinencephaly with a single cerebral ventricle is determined prior to 31-32 d.g. The teratogenetic period for pachygyria and lyssencephaly ends by 4 1/2 months of gestation and for polymicrogyria by 5 months of gestation.

(d) Cerebral malformations were classified into the following four major types and numerous subtypes:

Agenesis and hypoplasia
Dysgeneses
Developmental cyst formations
Congenital conditions associated with deformities of the CNS.

(e) Tests leading to the diagnosis of various congenital malformations of the nervous system were outlined. Particular attention was given to cortical dysgeneses as they are difficult to diagnose and frequently they underlie epilepsy and mental retardation.

(f) Among various factors leading to malformation of the nervous system listed are the chromosomal aberrations especially trisomy of 13-15 and 16-18.

(g) The discussion of importance of an early and comprehensive diagnosis and the outline of long range management concludes the study.

2. Extensive study was undertaken to provide analytical data on heparan sulfate which accumulates excessively in the tissue and is excreted in excess in the urine of Sanfilippo patients. Since there are certain clinical and biochemical inconsistencies, detailed chemical characterization of this compound was necessary. Acid mucopolysaccharides derived from the urine of heparan sulfate excretors (the Sanfilippo variant) and from mixed, dermatan sulfate and heparan sulfate excretors (the Hurler variant) were investigated. The molecular weight distributions by gel filtration and carbazole-orcinol (C/O) reactivities of the samples gave an average molecular weight of about one-third of normal for the Sanfilippo heparan sulfate which could be further separated into two fractions differing in molecular weight, sulfate content and primary structure. Optical absorption and rotatory properties from 250 to 185 mμ) of these glycosaminoglycans were studied to examine the conformational features of the accumulating substances. The results for the Sanfilippo heparan sulfate fractions indicate that two distinct heparin sulfates accumulate in this genetic defect, one with conformational structure essentially like that found in normal tissues but low in O-sulfate, the other with heparin-like conformation and high in N-sulfate content. The ultraviolet optical rotatory dispersion and circular dichroism of dermatan sulfate are greatly different

from that of heparan sulfate, providing an optical method of distinguishing among Sanfilippo and other Hurler variants.

3. Correlations of clinical, pathological and biochemical findings in certain neurological disorders are still scanty and there exists a need for clues to point the direction toward better oriented therapeutic and preventive measures. Seven brains of patients with cerebral palsy, epilepsy or metabolic disorders affecting the CNS were processed using large histological sections and by histochemical methods. Five of these were particularly valuable since they represent Unverricht epilepsy, cerebral lipidosis, one of Hurler, one of Hunter and one of Scheie type of mucopolysaccharidosis. The other two brains represented the syndrome of cortical dysgenesis and mental retardation. Some of the material used for biochemical studies was in the fresh state; the work is still in progress. The histological and chemical examinations of these six brains increased our collection and will be used for comprehensive analysis when sufficient number of cases is prepared.

4. Brain biopsy tissue of approximately 1-2 gm was obtained from six patients for diagnostic reasons. Cerebral cortex and white matter were separated and they are currently being processed for lipid, mucopolysaccharides and protein composition. In addition, histological and histochemical studies were made for the purpose of correlating the biochemical findings with pathological results.

Significance to Bio-Medical Research and the Program of the Institute: Detailed knowledge of pathology and pathogenesis of numerous conditions which underlie cerebral palsy, mental retardation and progressive cerebral degeneration is of basic importance for eventual therapeutic and preventive measures. Some of our studies in this field are of applied type and others here listed have basic implications. The disorders considered here are clearly the major responsibility of our Institute.

Proposed Course of the Project: This project has a wide scope and pertains to one of the major objectives of NINDS; therefore, it is considered as our permanent project.

Honors and Awards: None

Publications:

1. Dekaban, A.S.: Malformations of the Nervous System: Medical Aspects. In: Brennemann-Kelley Practice of Pediatrics. In press, 1971.

2. Stone, A.L., Constantopoulos, G., Sotsky, S.M., and Dekaban, A.S.: Optical rotatory dispersion of mucopolysaccharides. IV. Optical rotatory dispersion and circular dichroism of glycosaminoglycans and heparan sulfate fractions from the urine of patients with mucopolysaccharidosis (Hurler syndrome). Biochim. Biophys. Acta 222: 79-89, 1970.

Serial No. NDS(I)-63 SN/CN 1026(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical and Biochemical Studies on Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Kenton R. Holden, M.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: Currently in the U.S.A. there are about 2 million people who are or have been subject to epileptic attacks due to various causes. The onset of seizures is during childhood in over 65 per cent of these patients. Idiopathic (cryptogenic) epilepsy is the most common. Thus, there is a great need for broad as well as specific studies in the field of epilepsy, especially at the time of its onset. In the first place we must provide a better understanding of the underlying mechanisms of epileptic seizures and better treatment; this eventually should be followed by suitable preventive measures. Our present specific approach is employment of high fat diet for control of certain seizures during early childhood. Elucidation of the mechanism of its action is carried out in both the patients and in laboratory animals.

Methods Employed:

1. Clinical, developmental and neurological examinations and special tests.
2. Electroencephalographic studies.
3. Pneumoencephalography and arteriography when indicated.
4. Urinary and plasma amino acids.
5. Assays of carbohydrate metabolism.
6. Plasma lipid profiles.
7. Endocrinological assays when indicated.
8. Application of special therapeutic procedures including ketogenic diet.
9. Special studies in patients on high fat diet.
10. Determination of partitioned lipids in brain homogenates and subcellular particles of animals on high fat diet.

Patient Material: 14 inpatients (total patient days 327) and 5 outpatients. Most of the patients were suffering from "idiopathic" epilepsy. Experimental animals - rats on high fat diet.

Major Findings:

1. Four groups of weanling rats were maintained on 40% fat diet (normally rat diets contain 3-5% fat). The animals were sacrificed at predetermined intervals between 3-30 days after being on the high fat diet. The brains and livers were rapidly removed, immediately homogenized, and separated, using refrigerated ultracentrifuge, into four subcellular fractions: nuclei, mitochondria, myelin and synaptic vessicles with nerve endings. Determination of major classes of lipids was carried out in the plasma, liver and brain homogenates and subcellular fractions. The following tables summarize the main findings.

Table 1

Distribution of individual lipids in brain, liver and plasma in 51 day old rats established on 40% fat diet at weaning. Each value is a mean of separate determinations in 6 groups of 1-2 rats ± S.E. and expressed in mg per gram fresh tissue or per ml plasma.

		Cholesterol	Phospholipids	Triglycerides	Total Fatty Acids
Brain	Fat Diet	19.6 ± 1.6	49.6 ± 3.7	0.7 ± 0.12	29.3 ± 2.9
	Control	15.2 ± 2.0	43.4 ± 0.02	0.3 ± 0.02	22.5 ± 2.1
Liver	Fat Diet	26.3 ± 0.9	34.9 ± 2.9	29.4 ± 3.0	74.8 ± 4.2
	Control	2.8 ± 0.15	31.2 ± 1.6	4.6 ± 0.4	28.5 ± 1.6
Plasma	Fat Diet	1.37 ± 0.16	1.66 ± 0.12	1.63 ± 0.15	2.13 ± 0.1
	Control	0.94 ± 0.05	1.17 ± 0.08	0.83 ± 0.03	1.35 ± 0.13

It may be noted that there was a significant increase in cholesterol and phospholipid in the brains of animals fed high fat diet, as compared to control rats on regular diet ($P < 0.05$) whereas the overall increase in triglycerides was small. There was a higher content of total fatty acids in the brains of rats on high fat diet than in the rats on a regular diet; this was possibly related to a higher content of esterified lipids in the former animals.

The changes in liver lipids were quite different; the high fat diet induced increase in cholesterol of about eight-fold and of triglycerides about five-fold while the phospholipids showed no significant difference. The total fatty acids increased greatly paralleling the changes in the triglycerides and cholesterol. The most marked changes in the plasma lipids were about two-fold rise in triglycerides and total fatty acids. Plasma ketone bodies (not shown in Table 1) were four-fold increased and plasma glucose remained at 20% below normal controls. The high ratio of lipids per gram of liver and brain in relation to the lipid content per ml of plasma reflects higher water content in the plasma; this finding minimizes the possible contribution of blood lipids to the values of tissue lipids in analysis.

We have also followed progressive changes in the brain phospholipids and cholesterol in the weanling rats maintained on a regular and 40% fat diets at 3, 15 and 30 days. There was a gradual increase in both the cholesterol and phospholipids in the brains of rats on a regular diet during their period of growth between weaning and 50 days of age. In a comparable group of rats on 40% fat diet, the content of both the phospholipids and total cholesterol was consistently but not markedly higher than that in the control animals; the increase appeared to be highest at about 35 days of age when the animals had been on the high fat diet for about 15 days.

The weanling rats on 40% fat diet had a substantial increase in cholesterol and phospholipids in the myelin fraction and of phospholipids in the microsomal fraction. The remainder of cell fractions of animals on high fat diet had only slight or no significant increase in the lipid content.

Table 2

Distribution of cholesterol and phospholipids in brain subcellular fractions of 4 sets of rats (1-2 per group) on 40% fat diet and in controls on regular diet. All values in mg/g wet tissue.

	Myelin Small Fragm.	Synaptic Ves. & Nerve Endings	Mitochondria	Microsomes
Cholesterol				
Reg. Diet	2.84 ± 0.05	1.84 ± 0.03	0.56 ± 0.1	0.89 ± 0.02
Fat Diet	4.28 ± 0.12	2.85 ± 0.07	0.93 ± 0.03	1.31 ± 0.03
Phospholipids				
Reg. Diet	4.82 ± 0.26	8.21 ± 0.19	2.53 ± 0.09	2.85 ± 0.06
Fat Diet	6.51 ± 0.34	8.49 ± 0.07	2.94 ± 0.1	4.43 ± 0.11

It is possible that the increase observed by us in the cholesterol and phospholipid contents in the myelin fraction of adolescent rats fed high fat diet is related to the acceleration of myelin formation in the animals with persistently elevated plasma levels of cholesterol and phospholipids. Whether or not this increase in brain lipids will be maintained past the maturational stage remains to be shown. Also future study would have to include assessment of changes in cerebrosides.

2. Fifteen inpatients and twelve outpatients were investigated in detail and treated during the past 12 months. Nine of the inpatients had idiopathic or cryptogenic type of epilepsy and 7 of these were established on ketogenic diet. The remaining patients had various types of symptomatic epilepsy predominantly associated with tuberous sclerosis, certain new types of neurocutaneous syndromes (which are currently subject of special studies by this Section), Unverricht myoclonus epilepsy and others.

Of particular importance is an increase in our total number of patients treated with the ketogenic diet. After an addition of 8-10 more patients we will be ready to undertake statistical evaluation of the results and hopefully establish improved therapeutic guidelines over those which we published previously (Dekaban, A.S., Arch. Neurol. 15: 177-184, 1966).

3. Four patients with a specific variant of mucopolysaccharidosis (Scheie's type) were investigated clinically and using our newly developed biochemical technique. It was demonstrated that the group of patients who were reported in the literature to constitute a distinct syndrome, are in fact, afflicted by different nosological diseases.

Significance to Bio-Medical Research and the Program of the Institute. Our understanding of the pathogenesis of epileptic seizures and their control is still meager. Many young children become severely and permanently retarded as a result of frequent seizures. Biochemical approach to epilepsy is likely to provide us with better understanding of this syndrome complex and it may help us in the therapy of this distressing disorder. Present investigations enlarge the overall scope of the study on epilepsy which is one of the major research efforts of the Surgical Neurology Branch, NINDS. It also marks the beginning of epilepsy studies on the subcellular level.

Proposed Course of the Project: It is expected that this project will continue for many years.

Honors and Awards: None

Publications:

Constantopoulos, G., Dekaban, A.S. and Scheie, H.G.: Heterogeneity of disorders in patients with corneal clouding, normal intellect and mucopolysaccharidosis. Amer. J. Ophthal., in press 1971.

Serial No. NDS(I)-68 SN/CN 1523(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Lipid and Mucopolysaccharide Composition of the Cerebral White and Grey Matter.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.5
Other:	0.2

Project Description:

Objectives: Many of our patients with progressive cerebral degenerations have either excessive storage of a material in the nervous system (lipid, mucopolysaccharide, glycogen) or loss of structural material (diffuse sclerosis, subacute sclerosing encephalitis, axonal degeneration). Experimental animals will be used to establish biochemical techniques for analysis of partitioned lipids and mucopolysaccharides in subcellular fractions. Human material, both normal and abnormal, will be utilized principally as it becomes available from brain biopsies and at autopsy.

Patient Material: 16 inpatients (422 patient days) and 10 outpatients.

Methods Employed:

1. Rapid removal of brain from deceased patients with progressive cerebral degeneration or from those who are meant to be used as unaffected controls. Portions of the brain tissue are dissected and stored at -125°C for chemical studies.

2. Brain biopsies from patients with progressive cerebral degeneration and cerebrospinal fluid constitute the most valuable fresh human brain tissue.

3. Using recently available techniques, separation of neurons from glial elements and nerve fibers in the fresh tissue will be carried out.

4. Selected blocks of brain tissue are taken and fixed in appropriate solutions for histological and histochemical studies.

5. A modified chloroform-methanol technique of lipid extraction is utilized; this is followed by separation and identification of components by means of various columns and thin layer chromatography.

6. Extraction of acid mucopolysaccharides from brain and other organs using appropriate solvents as well as digestion with papain and pronase.

7. Assays of the involved enzymes in various genetic disorders using both artificial and natural substrates (collaborative approach).

8. Processing of the established fibroblast tissue cultures from respective patients for study of turnover of individual lipids and acid mucopolysaccharides using radioactive labels.

Major Findings:

1. Brain tissue obtained by biopsies and also at autopsy from the patients suffering from Hurler's and Sanfilippo's diseases were subject of extensive histological, biochemical, electron microscopic and biochemical studies. The result of these investigations significantly furthered our knowledge of the abnormal processes involved in the metabolism of the brain in these disorders.

2. The gross and microscopic abnormalities in the brains of Hurler and Sanfilippo variants of mucopolysaccharidosis are sufficiently marked to explain the progressive dementia and finally the decorticate condition of these patients. Examination of frozen sections treated with Sudan IV, Sudan black B, Baker's reagent and other stains showed distension of many neurons with excess of lipid staining substance. This leads one to expect a considerable increase in the brain lipid of the grey matter. Actual lipid determinations, however, do not substantiate this expectation.

BRAIN LIPIDS AS mg/100 mg DRY WEIGHT

	GREY MATTER			WHITE MATTER		
	Normal	Hurler	Sanfilippo	Normal	Hurler	Sanfilippo
Water (as % wet wt.)	80.4	84.3	80.1	73.9	80.1	79.3
Lower Phase Lipid	41.2	38.2	37.6	54.6	51.5	58.1
Cholesterol	9.6	9.0	6.7	15.7	12.9	13.7
Phospholipid	29.4	25.4	23.8	28.5	25.8	31.9
Phosphatidyl ethanolamine	8.2	6.7	7.2	9.7	8.4	9.4
Lecithin	11.0	9.9	9.5	8.4	7.8	11.1
Sphingomyelin	5.0	4.1	3.1	5.1	4.4	5.2
Phosphatidyl serine + inositol	5.2	4.7	4.1	5.4	5.1	6.1
Lipid Galactose	0.66	.90	1.11	2.68	2.20	2.97
Lipid Glucose	.037	.025	.315	.016	.017	.07
Lipid Galactosamine	<.01	.031	.119	<.01	<.01	<.01
Sialic Acid	.340	.498	.411	.038	.249	.096

Actually there is a decrease in lower phase lipids. Although the sugar lipids are increased in relation to normal values, their absolute amounts are not very large. To explain the obvious and marked increase of the lipid-staining substance as revealed by the histochemical methods, it is necessary to consider quantitative and qualitative changes in the grey matter cytoarchitectonics. Firstly, following longer duration of the disease the nerve cell population is considerably depleted. Secondly, not all neurons are affected to the same extent. Illustrations (in the published paper) show a better preservation of the nerve cells in the Vth than in the IVth lamina of the same section. Clearly, the only reliable assessment of the lipid content could be made on the isolated nerve cells from the affected patients and normal controls; this still poses formidable technical and other difficulties. By the cytochemical methods there was only a slight and variable increase of AMPS in the neural elements.

The early and rather severe involvement of the cortical neurons in the layers IV and II in the Sanfilippo variant correlates well with rapid mental deterioration of these patients, while their motor performance, which on the cortical level is dependent on the intactness of layers V and III, remains in a satisfactory state for a much longer time. The progressive loss of mental faculties in the Hurler's patients is generally much slower, especially in patients with onset of symptoms after 1-2 years of age.

3. The most striking abnormality in the grey matter of both the Hurler and Sanfilippo patients was the increased amount of glycolipids in the form of ceramide polyhexosides. Increased values of lipid galactose, glucose and galactosamine have not previously been reported in non-formalinized Hurler brain tissue. The only published report of lipid galactose was by Loeb *et al* who found a slight increase in a very young patient with the Hurler variant.

Marked increases in the ceramide polyhexoside partial breakdown products of gangliosides have, however, been found in Tay Sach's disease and G_{M1}-gangliosidosis. In these diseases which are characterized by accumulation of monosialogangliosides in both grey and white matter, the ceramide hexosides are increased only in the grey matter.

4. The amount of total ganglioside sialic acid in the grey matter of our patients was slightly but not significantly increased. This is essentially in accord with the reported cases of Hurler's syndrome for which grey matter ganglioside values are given. The total amount of white matter ganglioside sialic acid was increased by a factor of 2.5 in our Sanfilippo and by a factor of 8 in the Hurler patient. In Hurler's patients reported in the literature the white matter sialic acid was elevated 2-10 times the normal and in three the values were up to 1 1/2 times the normal; two of the latter were under four years of age. Thus far, no total values of ganglioside sialic acid of the white matter of the Sanfilippo variant have been published and the values in our patient are the first to be reported.

The distribution of brain gangliosides in Hurler patients have been described by a number of investigators. In most cases of Hurler syndrome and in our patients, G_{M2} and G_{M3} were increased in the grey matter, whereas in two cases of Suzuki's G_{M1} was increased although not to the extent found in our Sanfilippo patient. Only qualitative data on brain gangliosides in two cases of Sanfilippo has been reported by Wallace and his findings suggested an increase of G_{M2} and G_{M3}; this is in contrast to our quantitative results which showed increase in G_{M1} and decreased G_{D1a} in the Sanfilippo patient. In this respect it is interesting to note that our patient showed a slight to moderate increase of concentric membranous bodies which are characteristic for Tay-Sach's disease and the late infantile G_{M1}-gangliosidosis.

5. The listed data on the composition of the glycolipids and on the ganglioside sialic acid are of diagnostic and theoretical value since they provide a basis for tentative classification of disorders with abnormal lipid metabolism of the brain. From the results given in Table 3 (in original paper) it appears that the Hurler's patient has an increase in G_{M2} gangliosides (to a lesser extent than in Tay Sach's disease), while the patient with Sanfilippo variant has an increase in G_{M1} although this is less marked than in G_{M1}-gangliosidosis. Perhaps the above differences could also influence the varying extent of mental involvement in these two variants of mucopolysaccharidosis.

The several-fold greater content of the sialic acid in the white matter of the patients with Hurler's and Sanfilippo variants than in the normal controls may possibly be related to the diffusion of this substance from the broken down neurons and its accumulation in the viscous fluid which fills the greatly distended perivascular space.

Significance to Bio-Medical Research and the Program of the Institute: The composition and turnover of lipids and mucopolysaccharides in the brain in health and in various cerebral degenerations require concentrated effort. Our knowledge of the etiology and pathogenesis of these conditions is very scanty. Clinical procedures leading to modification of excretion or deposition of certain metabolites have to precede therapeutic approaches. This is clearly one of the important objectives of our Institute.

Proposed Course of the Project: This project is expected to continue for several years to come.

Honors and Awards: None

Publications:

Dekaban, A.S. and Patton, V.M.: Hurler's and Sanfilippo's variants of mucopolysaccharidosis. Arch. Path., in press 1971.

Serial No. NDS(I)-69 SN/CN 1655
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of the Composition and Biosynthesis of Cerebral Proteins in Experimental Animals and in Man.

Previous Serial Number: Same

Principal Investigator: Dennis F. Cain, Ph.D.

Other Investigators: Anatole S. Dekaban, M.D., Ph.D., J.M. Van Buren, M.D.

Cooperating Units: None

Man Years:

Total: 0.8
Professional: 0.8
Other: 0

Project Description:

Objectives: Brain proteins differ from those of other tissues particularly in the elevated levels of the acidic proteins. They are also rapidly synthesized and degraded - a phenomenon without a functional correlate.

An understanding of the role of these specialized aspects of protein metabolism in normal nervous tissue requires determination of the identity, composition and relative rates of synthesis and degradation, as well as the localization of individual proteins within various cell types and subcellular organelles. This information is particularly important in the case of the acidic proteins, some of which are known to be brain-specific.

The information thus obtained will allow an examination of the role of protein metabolism in certain neurological diseases.

Methods Employed: Working initially with the guinea pig, various protein fractions are extracted and purified by salt fractionation, isoelectric precipitation, gel filtration, ion exchange, chromatography, etc. These proteins are subsequently analyzed by electrophoresis in a soluble acrylamide gel system. The specific purified protein fractions are also characterized by amino acid analysis, sedimentation and immunological procedures.

In turnover studies, the brain proteins are labelled in vivo by intraventricular injections or in vitro (tissue slices) with ^{14}C- or ^{3}H-leucine.

Specific radioactivities of the individual protein fractions are determined by measurement of both protein and radioactivity after separation in the soluble acrylamide gel system which was devised for this work.

Evolving techniques for the separation of subcellular as well as whole cell units will continue to be evaluated for purposes of localization of the individual protein fractions.

Material: Brains and other organs of various small animal species will routinely be used. In addition, samples of brain and other nervous tissue obtained during surgery and at autopsy will be utilized.

Major Findings:

1. An acidic brain protein has been isolated from guinea pig. On electrophoresis in gel, this protein is fifth in electrophoretic mobility migrating well ahead of albumin. It is precipitated by 50 to 60% saturation with ammonium sulfate. It elutes from DEAE-cellulose column in a pH and NaCl gradient at pH 7.2 and 0.18 M NaCl virtually free of other contaminants. Preparative electrophoresis yields a material which moves as a single band. Gel filtration with marker proteins indicated a molecular weight of about 74,000. In the analytical centrifuge, it moved as a single symmetrical peak and calculations based on sedimentation velocity and diffusion showed a molecular weight of 86,000. Under dissociating conditions, however, chromatography on Biogel A 5m indicated a molecular weight of 42,000, suggesting that the native molecule is composed of two subunits. The ultraviolet absorption spectrum gave no indication of a prosthetic group. On amino acid analysis, a high content of aspartic and glutamic acid was found with a ratio of acidic to basic amino acid of 1.4 The high electrophoretic mobility is apparently due to the high content of glutamic and aspartic acids since chemical and enzymatic analysis failed to demonstrate significant amounts of sialic acid. The protein is stable in brain post mortem and turns over at about the same rate as the other acidic proteins.

2. Fresh human brain tissue obtained by diagnostic biopsy or during therapeutic procedures was examined by histological and chemical procedures. Efforts were made to correlate the microscopic findings with spectrum of soluble proteins obtained by electrophoresis of separate extracts of white and grey matter on acrylamide gel. The extracted proteins were separated into about 20 components, six of which migrate more rapidly than albumin.

Microscopic examination of five temporal lobectomies which had been performed for psychomotor epilepsy showed normal structure of the cortex and white matter in three, and a slight decrease in glial elements and occasional neuronophagia in the other two. Gel patterns of the brain proteins of the first three patients were very similar with small characteristic differences between white matter and cortex. The gel pattern of the protein from the white matter of an advanced case of subacute panencephalitis (characterized by dense gliosis) showed a very prominent component centered at band 16, while in the cortex bands 7 and 11 were very heavy. In an astrocytoma (grade III) microscopically characterized by the presence of astrocytic and spongioblastic elements, bands 15 and 20 were prominent with virtual absence of the acidic proteins 1, 3 and 5. The protein pattern in specimens from another glioma, a cerebral lipidosis, and Sanfilippo's disease was not remarkably changed.

Significance to Bio-Medical Research and the Program of the Institute: There is some suggestive and still indirect evidence that specific cerebral proteins may be involved in the processes of memory and intellectual functions. There are large numbers of subjects with undifferentiated type of mental retardation who defy all investigative approaches in clarifying the underlying abnormality. Because of several technical difficulties the brain proteins have not been thus far explored in these and many types of other patients. The availability to us of surgical and post-mortem tissue of a number of hitherto obscure neurological diseases provides us with unique opportunity to study cerebral proteins in these patients. Greater understanding of these conditions is one of the research responsibilities of our Institute.

Proposed Course of the Project: This project will continue for several years.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-57 SN/CP 401(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Psychological Evaluation of Temporal Lobe Operations

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. M. Van Buren, M.D., Ph.D. and A. Ommaya, F.R.C.S.

Cooperating Units: None

Man Years
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objectives: To study neurosurgical patients with temporal lobe disorders with regard to intellectual abilities, visual perception, linguistic functions and other "personality" features.

Methods Employed: Intelligence, personality and other specialized verbal tests; tests of visual perception. A test of "stereotypy"--the ability to approach "random" movement--records 5-minute sessions.

Major Findings: Analysis of results with a "Rectangle-Cross-Triangle" (RCT) test may have provided some further perspective on the nature of the visuomotor factor reported on recently with the "Square-Triangle" (ST) test. The ST test involved assembling the same 4 blocks into these two forms 10 times alternately; it measured mostly the traditional visuomotor factor in the Wechsler-Bellevue intelligence test, and seemed to be unrelated to the closure factor in visual perception. The RCT test involves assembling the 3 forms out of 5 blocks; the patients were asked to assemble each form 5 times inside frames and then attempt to assemble the forms 5 times each without the help of the enclosing frames. In the latter half of the test a time limit of 3 minutes was allowed before use of the appropriate frame was required. As expected, many patients even after the practice were unable to assemble the forms before the time limit without the frames; sometimes a patient took longer than on the first trial with the frame. Two specific perceptuomotor problems appeared to exist: a) with the triangle the longest side of the largest block was often placed inappropriately as the base, b) with the cross, one of the smallest pieces was often used inappropriately to fill in a nick in the largest piece. The problem with the triangle occurred both with and without the frame; the problem with the cross occurred only without the frame. Because of these perceptual problems, it is hoped that factor analyses of these data will enable some link to be shown between the RCT and closure tests (perhaps only in male patients). Minor sex differences not reported in the previous article suggest the sex differences might be fruitful to investigate in the possible relation of RCT to closure.

An opportunity arose this year to analyze some data from brain-injured patients at Walter Reed General Hospital (in cooperation with Major Frederick Smith of the Psychology Service in their Department of Psychiatry and Neurology). The main point of the investigation was to relate time since injury to factor scores on the Wechsler Adult Intelligence Scale (WAIS); in comparison with the older NINDS patients with epilepsy and movement disorders, their scores might provide better perspective on the presumed nature of normal brain function, and the data on these younger patients might illuminate the course of their quicker recovery. Scores were tabulated for 268 subjects tested during the years 1965-1970; most cases with missile wounds were injured in SE Asia and most accident cases were injured in the US. A total of 117 cases were deemed to have sufficiently lateralized damage to enable the usual comparison between left- and right-hemispheric dysfunction. The factor analysis of the total group produced 3 conventional factors of verbal comprehension, visuomotor skill, and numerical ability; application of Horst's general factor solution produced a single and appropriate factor. The left-right difference was clear for the verbal and visuomotor factors except in the case of frontal-lobe damage. A just-significant correlation was obtained between days since injury and both verbal and general factors, and, surprisingly, it seems to be independent of side of injury. A reanalysis of this data with Jöreskog's maximum-likelihood methods might simplify some of the results or improve them; unfortunately, difficulties have arisen in adapting his two programs from the CDC to the IBM computer.

Along with the WAIS data, scores for the Wechsler Memory Scale were obtained on 214 of the WRGH patients. Data for 754 of our patients on this test have also been assembled for factor analysis. In both groups it appears that the memory subtests can be understood in terms of the 2 main Wechsler factors plus another small memory factor; it is assumed that a means of producing 2 general factors by the Horst technique will readily be found. In any event, the probable relationships to lateralized memory factors will be investigated.

Arrangements are being developed with the Perinatal Branch to investigate the effects of lateralized early damage (spastic hemiparesis, focal motor seizures) on the WISC (Wechsler Intelligence Scale for Children) data being obtained at 7 years of age. The hope is that the data someday may serve to check for similarities to previously reported results (1969) showing paradoxically that early left-hemisphere damage, although capable of leading to right-hemisphere speech in adulthood, has less effect on higher verbal functions than on nonverbal intellectual functions.

The first clear post-operative deficit with right temporal removals was reported by Milner (1958) for the McGill Picture Anomalies Series. In 1960 Shulman failed to confirm the phenomena, but Milner recently (1970) reconfirmed her results with a large number of cases subdivided into several different levels of intelligence. An analysis of our data on 18 cases confirms her results; the clarity of this phenomena probably depends on careful selection of cases such as was done to produce our small group. Some factor analyses are being performed on large groups of results, but, as yet, no insight has been provided as to the factors involved in picture anomalies--i.e., it has not proven to be related to the closure factor.

Significance to Bio-medical Research and the Program of the Institute: With factor analysis of data on large groups and careful selection of surgical patients, relations between factor scores and cerebral lesions can be established selectively for different loci and for each sex; these relationships have implications for the physiological analysis of the "higher mental" processes.

Proposed Course of Project: Further factor analytic work on psychometric results, publication of findings.

Honors and Awards: Fellow, American Psychological Association (Physiological & Comparative Division)

Publication:

Lansdell, H.: Relation of extent of temporal removals to closure and visuomotor factors. Percept. Mot. Skills 31: 491-498, 1970.

Serial No. NDS(I)-63 SN/CP 1032(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Psychological Effects of Subcortical Lesions Used for Relief from Abnormal Movements.

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. Van Buren, M.D., Ph.D., and A. Ommaya, F.R.C.S.

Cooperating Units: None

Man Years
    Total: 0.0
    Professional: 0.0
    Other: 0.0

Project Description

Objectives: In comparison with the effects of other forms of neurosurgery, to investigate the changes resulting from subcortical lesions with the aim of delineating subcortical from cortical factors in brain function.

Methods Employed: A battery of tests [the same as those used in Project Serial No. 401(c)] has been used on patients before, two weeks after, and again a year or more after operation.

Major Finding: No further analyses limited to this type of surgery were done this year; the data obtained from this type of patient were incorporated into the analyses performed in Project Serial No. 401(c). Some analyses in future will examine the specific effects of this type of surgery on the factors being determined from the battery of tests.

Significance to Bio-medical Research and the Program of the Institute: Future differentiation of memory, perceptual, and verbal factors in the battery will provide an opportunity to differentiate the factors physiologically by comparing the effects of these disorders (and operations) with the effects of the largely cortical disorders (and removals).

Proposed Course of Project: Further analysis of the psychometric data.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 SN/CP 1033(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Phonemic Aspects of Dysnomia

Previous Serial Number: SAME

Principal Investigators: Herbert Lansdell, Ph.D. and Paul Fedio, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 0.0
Professional: 0.0
Other: 0.0

Project Description

Objective: To investigate the phonemic aspects of induced dysphasia.

Methods Employed: Filmstrips and a projector are used. The filmstrips contain three orders of pictures; the names of the pictured objects have a wide range of phonemes in the initial and final positions. The nouns have a high frequency in the English language. There is automatic control of exposure time.

Major Findings: Data solely relevant in this project are being accumulated with its use during carotid Amytal tests. To date, there appears to be no confirmation of the previous result regarding the influence of the initial and final phonemes on naming errors; the nature of the word substitutions unfortunately cannot be investigated with the present small number of observations.

Significance to Bio-medical Research and the Program of the Institute: Future different types of data may continue to extend our knowledge of the phonemic aspect of speech and delineate this clue with regard to the physiological mechanisms of speech and verbal activity.

Proposed Course of Project: The phonemic aspect of results will be analyzed when sufficient data are obtained.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 SN/NR 925 (c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Small Sella Turcica

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Thomas Aceto, Jr., M.D.
Mary Parker, M.D.
Alvin Hayles, M.D.
Colin B. Holman, M.D.

Other Investigators: Doris A. Sadowsky

Cooperating Units: The Children's Hospital, Buffalo, New York

The National Pituitary Agency, Baltimore, Maryland

Washington University, St. Louis, Missouri

The Mayo Clinic, Rochester, Minnesota

Office of Biometry, NINDS, NIH

Man Years:

Total:	.1
Professional:	.1
Others:	.0

Project Description:

Objectives: An anatomo-radiographic study to confirm in a large series of patients the diagnostic significance of the small sella turcica. In addition, we plan to establish whether or not the sella turcica of hypopituitaric dwarfs changes in size after growth hormone treatment.

Methods Employed: Volumetric studies of the sella turcica in many hypopituitaric dwarfs before and after growth hormone treatment.

Major Findings: During the past year, additional sella turcica measurements in various types of dwarfs have been gathered.

The "small sella turcica" is a diagnostic entity of sufficient specificity to be valuable for the differential diagnosis of hypopituitary vs. other types of dwarfism.

A preliminary analysis of sella turcica volumetric measurements in hypopituitary dwarfs subjected to a first treatment course with growth hormone, has failed to show changes in the sella turcica size during the follow-up period of many months up to a maximum of two years. This is in contrast with the linear growth of these patients which has definitely been stimulated by the administration of human growth hormone.

Significance to Bio-Medical Research and the Program of the Institute: The rarity of the small sella turcica in the average hospital population and in non-hypopituitaric dwarfs, and the frequency of this radiographic finding in hypopituitaric dwarfs make this x-ray sign a valuable diagnostic element. The diagnosis of idiopathic hypopituitaric dwarfism in pre-pubertal children is difficult and dependent to a large degree upon indirect chemical estimations of various trophic hormone deficiencies of the pituitary. The finding of a sella volume below the normal range for the patient's age should suggest and should be considered as confirmatory evidence of hypopituitarism in the doubtful cases.

The absent modification of the sella turcica size in hypopituitary dwarfs after treatment with growth hormone, has probably some theoretical significance in relation to the mechanism of therapeutic action of the injected hormone. This lack of change is particularly interesting because it contrasts with the significant linear growth observed in the treated dwarfs.

Proposed Course of Project: This project has been terminated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 SN/NR 1047(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Gerald L. Schall, M.D.
Steven M. Larson, M.D.
William L. Ashburn, M.D.
Sherman C. Stein, M.D.
Ayub K. Ommaya, F.R.C.S.

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Radioisotope Laboratory, University of California, San Diego, California

Man Years:

Total:	.4
Professional:	.4
Others:	.0

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained.

Methods Employed: The radioisotope cisternography and ventriculography procedures are now well established.

Recently, we have carried out dual, sequential, radioisotopic scintigraphy of the brain and the CSF cavities. This technique allows the visualization of both the brain and cisternal spaces (encephalo-cisternography) or the brain and ventricular system (encephalo-ventriculography). Display of the gathered dual-radioisotopic data is accomplished in two different colors, so that the anatomical features of the adjacent anatomic compartments (brain and CSF cavities) will stand out vividly.

Major Findings: During the current fiscal year we have gathered additional experience in the following areas:

1) Radioisotope encephalo-cisternography and radioisotope encephalo-ventriculography which have proven very valuable for the evaluation of the relative size of brain substance vis á vis the CSF cavities and for the differentiation of extra- versus intra- cerebral endocranial lesions.

2) An analysis of the influence of previous lumbar puncture and pneumoencephalography on the incidence of unsuccessful radioisotope cisternography has been carried out. This study indicates that previous lumbar puncture and pneumoencephalography, per se, are not, (as it was previously incorrectly presumed) important predisposing factors to unsuccessful intrathecal injection in cisternography. Rather, the inadequate injection may be directly related to the technique at the time of the puncture itself.

3) Additional experience has been gathered with cisternography in cases of porencephaly.

4) Radioisotope cisternography and radioisotope ventriculography have been carried out in a number of normal and hydrocephalic dogs in an attempt to establish an experimental model of communicating hydrocephalus.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we have now a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoidal pathways.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Honors and Awards: None

Publications: Di Chiro, G.: Radioisotope cisternography in hydrocephalus. New Eng. J. Med. 284:54, 1971.

Di Chiro, G.: Radioisotope cisternography, ventriculography, and myelography. In Gilson, A. J. and Smoak, W. M., III: Central Nervous System Investigation With Radionuclides., Springfield, Illinois, Charles C. Thomas, Publisher, 1971, 310-315 and 316-326.

Di Chiro, G.: Scanning of cerebrospinal fluid cavities. To be published in the Proceedings of the "Symposium on Cerebrospinal Fluid" held at the XIV Congreso Latinoamericano de Neurocirugia, March, 1971, in Punta del Este, Uruguay, (In press).

Serial No. NDS(I) 65 SN/NR 1195(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Selective Arteriography of Spinal Cord Arteriovenous Aneurysms

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Jean R. L. Herdt, M. D.
Ayub K. Ommaya, F.R.C.S.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Pathologic Anatomy Branch, National Cancer Institute, NIH

Man Years:

Total:	.2
Professional:	.2
Others:	.0

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964, we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels.

Major Findings: During the current fiscal year we have been able to further develop the technique and to augment our experience with selective arteriography of the spinal cord. In particular:

1) The observation has been made that all cases (three) of documented spinal subarachnoid hemorrhage in our series of over 50 verified arteriovenous malformations of the spinal cord had co-existent saccular (berry) arterial aneurysms. In turn, every case (four) presenting combined saccular aneurysms had direct or indirect (clinical symptoms as the "coup de poignard") evidence of spinal subarachnoid bleeding. Spinal hemorrhage, on the other hand, was never observed in any of the spinal cord arteriovenous malformations without concomitant arterial aneurysms. We conclude that the presence of a saccular arterial aneurysm greatly predisposes to a spinal subarachnoidal bleeding, and perhaps is a necessary prerequisite for the occurring of this hemorrhage.

2) Further experience has been gathered in the area of tumoral cord lesions. The validity of the previously reported differential angiographic diagnostic criteria between hemangioblastomas and arteriovenous malformations of the spinal cord has been confirmed. A new, important finding has been that in cases of intradural, extramedullary spinal cord tumors (neuromas, meningiomas), characteristically spiral (corkscrew) shaped dural vessels may be demonstrated. The presence of these enlarged dural vessels is of differential diagnostic significance. These vascular channels are not, in fact, found in intramedullary spinal cord lesions.

3) The "directional" blood flow in the spinal cord arteries has been the subject of our extensive investigation. Ascending and descending blood flow currents do exist in the anterior and posterior spinal cord arteries in man. The direction of these currents varies in normal cases in the various sections of the spinal cord arteries. Blood flow from two adjacent radicular arteries develops in the anterior spinal artery in opposite (converging) directions. "Watershed" areas do exist. Reversals of blood flow currents in the anterior spinal artery in pathological conditions occur quite frequently in man and can be demonstrated angiographically. The appraisal of the blood flow direction in the spinal cord vessels is diagnostically important. It is also conceivable that reversals of blood flow directions in the spinal cord arteries can cause "steal phenomena."

4) We have, now, definite evidence that spinal cord angiography is the diagnostic tool of choice in cases of suspected obstructive vascular disease of the spinal cord. A group of patients with clinical

diagnosis of obstructive vascular disease of the spinal cord has been studied by selective arteriography. In five instances the angiographic findings have supported this diagnosis. The findings include "beady" or abnormally thin and tortuous anterior spinal artery, presence of collateral circulation, and inpingement on the anterior spinal artery by an intervertebral disc.

5) Our angiographic experience using the selective arteriographic technique in cases of arteriovenous malformations of the spinal cord has been significantly augmented. Our present series includes more than 50 patients with such a condition.

Significance to Bio-Medical Research and the Program of the Institute: Selective arteriography of the spinal cord is increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Further technical improvement of the selective arteriography of the spinal cord lesions. We are particularly interested in radiographic magnification using the new fractional focal spot x-ray tubes. The resolution of the small vessels should be improved with these magnification techniques.

Angiotomography is another development which we are "watching" and considering for the spinal cord studies.

Improved x-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following, very closely, the recent developments in the area of polimeric iodinated x-rays contrast media (dimers and trimers of various basic molecules).

We hope to extend our clinical experience to the selective arteriography of the spinal cord applied to the acute traumatic spinal cord injuries. In an important, recently published monograph "INJURIES TO THE CERVICAL SPINE AND CORD IN MAN, by B. R. Selecki, overwhelming experimental, clinical, and post-mortem evidence is offered regarding the importance of the vascular components in the cord injuries. Selecki states "An important degree of vascular damage and swelling must become the main problem to be solved in the future." Obviously, spinal cord angiography will have to play an important role in the future research devoted to the traumatic injuries of the spine.

We have started, and we are planning to expand, microangiographic studies of the embryonic and fetal human spinal cord. This will enable us to better understand certain anatomical and developmental aspects of the spinal cord vessels.

Finally, related to the area of the arteriography of the spinal cord are our post-mortem studies in "supine myelography." Prone myelography is the technique currently used in clinical material. This should be complemented with supine myelography for a better demonstration of various intrathecal anatomical structures (particularly meningeal and vascular). When a better understanding of the normal and pathologic "defects" in the supine myelography will be reached, this will improve the diagnostic possibilities of myelography, and, in addition, will permit a better use of spinal cord angiography.

Honors and Awards: None

Publications: Di Chiro, G.: The development of spinal cord angiography. Acta Radiol. (In press).

Di Chiro, G.: Angiographic evaluation of spinal cord vascular disease. In Boggs, R. P. (Ed): Proceedings of International Conference on Vascular Disease of the Brain and Spinal Cord., New York, New York, Wiley Interscience Co., (In press).

Di Chiro, G. and Fried, L. C.: Blood flow currents in spinal cord arteries. Neurology (In press).

Serial No. NDS(I)-66 SN/NR 1283(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Empty Sella Turcica

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Endocrinology Branch, National Cancer Institute, NIH

Pathologic Anatomy Branch, National Cancer Institute, NIH

Man Years:

Total:	.1
Professional:	.1
Others:	.0

Project Description:

Objectives: During previous anatomic work on the sella turcica, "empty" sellae, i. e. instances in which the pituitary may so far fail to fill the sella that the anatomic specimen appears empty, were observed in several cases. The empty sella is usually larger than normal: In fact, in a series of 66 postmortem anatomic observations of the sella turcica and its content, the largest sella was an "empty" one. A selective hydrocephalus of the intrasellar subarachnoid space is considered to be the cause of the large empty sella.

The existence of a large empty sella has pragmatic importance. A large empty sella may mimic an intrasellar tumor and a differential diagnosis has to be made. In addition, the abnormal dilatation of the intrasellar subarachnoid space may cause, probably with a pressure mechanism, a hypopituitaric syndrome (as in several of our cases) or the development of an intrasellar tension cyst. These cysts may have eroding ability resulting in a break of the sellar floor and an ensuing cerebrospinal fluid rhinorrhea. The empty sella acquires, therefore, significance as a specific pathologic entity. Finally, the knowledge of the existance of a large intrasellar subarachnoid space is important if therapy of the pituitary gland (whether trans-sphenoidal hypophysectomy, trans-sphenoidal ^{90}Y interstitial therapy, or external irradiation) is contemplated.

Methods Employed: Suitable views (hanging-head lateral with autotomography or zonography) are taken at the end of most pneumoencephalographies. Prior to taking these special views the patient is kept in the hanging-head position for at least five minutes, the intent being to fill with air the intrasellar subarachnoid space if present. Particular attention is paid to patients with large sellae and with "dyspituitarism." Occasionally, surgical or autopsy follow-up is obtained.

In selected patients with an empty sella syndrome, radioisotope cisternographic studies are carried out to better evaluate the "dynamic" relationship of the cisterna intrasellaris and the rest of the subarachnoidal space.

Major Findings: Moderately large to large or very large intrasellar subarachnoid space (cisterna intrasellaris) has been observed in an unexpectedly high number of patients with normal, borderline big, or big sellas. The intrasellar subarachnoidal space is located in the anterior part of the sella, with the pituitary gland occupying the infero-posterior part of the sellar cavity. In certain instances the compressed pituitary gland appears to be reduced to a shell-like structure filling only the postero-inferior section of the sella.

Significance to Bio-Medical Research and the Program of the Institute: The knowledge of the large empty sella is important for: a) the differential diagnosis of intrasellar tumor; b) the explanation of certain hypopituitaric syndromes and certain types of spontaneous cerebrospinal fluid rhinorrhea; and c) to avoid mistakes when contemplating trans-sphenoidal (surgical and ^{90}Y) or external radiation pituitary treatment.

Proposed Course of Project: This project has been terminated.

Honors and Awards: None

Publications: Di Chiro, G.: An Atlas of Detailed Normal Pneumoencephalographic Anatomy. (Second Edition). Springfield, Ill., Charles C. Thomas, 1971, 343 pp.

Serial No. NDS(I)-67 SN/NR 1413(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Floro Miraldi, M.D., Ph. D.
Eugene T. Yon, Ph. D.

Other Investigators: None

Cooperating Units: Department of Nuclear Medicine, Clinical Center, NIH

Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio

Man Years:
Total: .1
Professional: .1
Others: .0

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space-occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if an effective and efficient method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintigraphic record of the head would be attained by tomography. We have been theoretically analyzing the various methods tried by other investigators to solve the problem of scanning tomography. These methods fall into four general catagories: 1) focusing collimator systems; 2) systems using collimators inclined to the plane of interest; 3) scanning with collimators in the plane of interest; 4) time-of-flight positron systems.

We have performed a mathematic appraisal of the three fundamental parameters: Sensitivity, resolution, and efficiency.

We have proposed a new approach: The strong focusing multi-layered Tomoscanner. Judging from theoretical considerations, this new system holds promises to be, in many ways, advantageous compared to the ones already tested by other investigators.

Major Findings: The prototype of the Tomoscanner is in advanced stage of preparation. It is anticipated that the new equipment will be on the NIH premises, during the spring of 1971. A large room in the Department of Nuclear Medicine, Clinical Center, is being readied for the installation of this new machine.

Significance to Bio-Medical Research and the Program of the Institute: A successful and simple method of scanning tomography would revolutionize the field of neuroradiology, and parenthetically other fields of radiology. Brain scanning and brain scintiphotography are in fact essentially innocuous (no mortality or morbidity), causing no discomfort to the patient, "less radiation giving" (if proper isotopes are used), and cheaper than other neuroradiologic procedures. Brain scanning and brain scintiphotography are also simple, quick procedures and may in fact, in most instances, be carried out on an ambulatory basis without admitting the patient to the hospital. Last but not least, interpreting a brain scan or a scintiphoto is much simpler and straight-forward than reading a cerebral angiogram or a pneumoencephalogram. If, therefore, the resolution of brain scanning can be significantly improved by tomography, a complete change-over of the neuroradiology departments will occur. Brain scanning and brain scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by any of our diagnostic neuroradiologic methods, would then probably be "picked up." An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods taken as a "whole" would then occur.

Proposed Course of Project: The Tomoscanner prototype will soon be tested in clinical material. Particular attention will be devoted to patients with brain tumors and other endocranial space occupying lesions. The Tomoscanner will also be evaluated in conjunction with the techniques of radioisotope cisternography and radioisotope ventriculography.

A limited amount of work with tomographic scanning in experimental models (dogs with brain tumors and hemorrhagic lesions) is also anticipated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/NR 1654(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Spinal Cord Angiography

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Larry C. Fried, M.D.
John L. Doppman, M.D.

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Department of Neurosurgery, University of California School of Medicine, Davis, California

Man Years:
Total: .2
Professional: .2
Others: .0

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established. It is obvious, however, that two conditions must be met if this method is to become a more advantageously and frequently used diagnostic procedure. The technique needs to be simplified, and its clinical indications must be expanded to include the common and important obstructive vascular disease. To attain the two above-mentioned goals, we have started to work in the area of experimental spinal cord angiography.

Methods Employed: Experimental spinal cord angiography has been carried out in various animals: Primates--monkeys and chimps,--dogs, cats, rats. Our largest experience, however, has been gathered with the Rhesus monkey (Macaca mulatta): Over 100 such primates have been used for our work. The remarkable similarity of the anatomical arrangement of the cord arterial supply in the macaque and man makes this species of monkey particularly suitable for this type of research. We have been centering our attention on the thoracolumbar cord, although few experiments have been carried out to angiographically evaluate the cervico-thoracic cord.

The angiographic studies were performed by aortic catheterization. Serialograms were taken in both anteroposterior and lateral projections, and in most instances a direct radiographic magnification technique was used (tube with 0.3 focal spot). Three basic aortographic techniques were used: 1) Conventional, 2) Pressor amines aortography, where the angiographic studies followed the aortic injection of various pressor amines, 200 mcg. of levarterenol base being most frequently used, and 3) External abdominal compression aortography where the angiographic study was carried out after establishing marked (200 to 300 mm of Hg.) external abdominal compression by means of an inflatable balloon.

The angiographic studies have been followed in a large group of animals by surgical exposure and selective ligation of the radiographically visualized vessels. Surgical occlusion of the arteries of Adamkiewicz and of the anterior spinal artery immediately above and immediately below the origin of the Adamkiewicz has been accomplished in many monkeys. Clinical, radiographic and postmortem follow-ups of the operated monkeys have been obtained. The anatomo-radiographic observations made at angiography, and the experimental lesions caused by the vessels ligation at surgery, have been matched and controlled with the clinical, gross pathological, microangiographic, and fluorescent substances perfusion techniques. Blood flow "currents" within the large spinal cord arteries have been evaluated by visual and cinematographic controls of the surgically exposed cord after perfusion with vital dyes (coomassie blue, Evans blue).

Major Findings: During the current fiscal year, we have been able to:

1) Demonstrate angiographically--using the previously reported techniques of pressor amine aortography and abdominal compression aortography--the normal spinal cord veins in the Rhesus monkey (Macaca mulatta).

2) The demonstration of the normal veins, has in turn allowed us to define an angiographic circulation time (ACT) for the spinal cord vasculature. ACT is the time interval from the opacification of the artery of Adamkiewicz (arteria radiculo-medullary magna) to the visualization of the vena radiculo-medullary magna. At present, we are trying to eliminate as much as possible artifactual elements from this determination. For this purpose, we are concentrating our attention on the abdominal compression aortograms, where, in contrast to the pressor amine aortography, no systolic blood pressure elevation is observed. Preliminary tabulation of the available data would

indicate that the ACT in the Rhesus monkey is of about two seconds

3) By the previously described technique of visual and cinematographic controls of the surgically exposed cord after perfusion with vital dyes (coomassie blue, Evans blue), we have gathered clear evidence that ascending and descending currents of the blood flow do, indeed, exist in the large spinal cord arteries of the Rhesus monkey.

Significance to Bio-Medical Research and the Program of the Institute: Our results with experimental spinal cord angiographic procedures offer the basis for attempting to develop similar spinal cord angiographic techniques in man. These techniques would, in turn, allow a more careful clinico-radiographic analysis of many pathological conditions of the spinal cord.

Normal spinal cord veins in man are not easily demonstrable. Some preliminary results would indicate, however, that this is not an impossible task. If demonstrable, the venous phase of the spinal cord angiography in human patients could be used for diagnostic purposes. This would obviously increase the amount of information which we may obtain from the arteriography of the spinal cord which, at the present time, is essentially only informative in the arterial phase.

The determination of the normal cord circulation time, if possible in man, would also represent an important diagnostic parameter.

The blood flow currents in the spinal cord arteries, and reversals of these currents, have great significance in the vascular pathophysiology of the spinal cord. These currents are, in our opinion, one of the basic compensatory mechanisms in cases of sectional cord ischemia.

Proposed Course of Project: We plan to gather further experience with the techniques of pressor amine and compression aortography. In particular, we are going to try new types of pressor amines, different dosages, and "cocktails" of the various drugs. We also intend to test the usefulness of some "protective" substances (glucose, mannitol, dextran, procaine). Various modifications of the compression technique will be tried. When we are convinced of the reasonable safety of these new experimental techniques, we will start to apply our acquired knowledge to human clinical material.

Honors and Awards: None

Publications: Fried, L. C., Doppman, J. L., and Di Chiro, G.: Direction of blood flow in the primate cervical spinal cord. J. Neurosurg. 33:325-350, 1970.

Fried, L. C., Doppman, J. L., and Di Chiro, G.: The venous phase of the spinal cord arteriogram. Acta Radiol. (In press).

Serial No. NDS(I)-70 SN/NR 1791(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Angiography of Cerebral Venous Obstructive Disease

Previous Serial Number: None

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: None

Cooperating Units: Experimental Surgery and Clinical Medicine, Laboratory Aids Branch, Division of Research Services, NIH

Man Years:

Total:	.1
Professional:	.1
Others:	.0

Project Description:

Objectives: "Stroke" is one of the pathological conditions which recently has been a target attacked from many different sides. For instance, in the last ten years, a great amount of research has been dedicated to the angiographic evaluation of the cerebrovascular obstructive disease. The major emphasis, however, has been put on the arteriographic findings. A perusal of the literature clearly shows that only scattered observations have been made regarding the phlebographic changes. The neuropathological studies (Hahn, Loeb, Zülch), on the other hand, have pointed out the fact that the obstructive venous pathology, although not as frequent as its arterial counterpart, represents an important nosologic entity.

We intend to explore the angiographic changes occurring in the cerebral vessels after experimental obstruction of the various brain venous channels (veins and dural sinuses) in a primate experimental model. We are particularly interested in establishing, on the basis of these experiments, which venous occlusions can be well tolerated and which are, instead, incompatible with good function. We will try to establish, again angiograpically, what types of compensatory mechanisms take place after the venous ligations. Do new venous draining pathways open, and if so, which and how efficient are these reserve discharge routes? The angiographic work will be carried out in close connection with an analysis of the clinical status of the operated animals.

The knowledge acquired in our experimental models will be extended to human material.

Methods Employed: Catheter arteriographic studies in the monkey (Macaca mulatta) are performed before and after ligation of one or several of the large endocranial venous channels.

Together with the post-surgical angiographic evaluation, an analysis of the clinical condition of the monkeys is carried out. After various periods of time following the venous ligations, the animals are finally sacrificed in order to carry out microangiographic and gross and microscopic neuropathologic studies.

We are planning micrangiographic studies of brains (supplied by the AFIP) of patients who have died of possible cerebral thrombophlebitis.

Major Findings: In this early stage of our research, we are acquiring experience in the evaluation of the monkey cerebral angiography. We have been impressed by the remarkable similarity of the major, and even medium size vessels (arteries, veins, dural sinuses) in the Rhesus monkey and man. We have good evidence, therefore, that the primate model will be applicable to the goals of our research project.

We have already carried out in a group of Rhesus monkeys, ligation of the superior longitudinal sinus in its anterior, middle, or posterior third. Cerebral angiographic studies have been performed before and at various time intervals after the ligation. We are now in the process of evaluating our angiographic, surgical, gross and light microscopic anatomo-pathologic findings in the operated monkeys.

Significance to Bio-Medical Research and the Program of the Institute: This project can increase our understanding of the pathophysiology of the cerebrovascular occlusive disease. Possibly, we will be able to recognize and separate specific conditions (mainly due to venous drainage impairment) in the large group of pathologic entities which are classified under the heading of "stroke."

Proposed Course of Project: We intend to continue our experimental investigation in monkeys.

Later on we intend to apply the acquired experimental knowledge to the human clinical experience.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 SN/NR 1865(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Scanning (and Tomoscanning) of Tumoral and Hemorrhagic Experimental Cerebral Lesions.

Previous Serial Number: None

Principal Investigators: Giovanni Di Chiro, M.D.
Sherman C. Stein, M.D.

Other Investigators: None

Cooperating Units: Department of Nuclear Medicine, Clinical Center, NIH

Experimental Surgery and Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services, NIH

Man Years:
Total: .3
Professional: .3
Others: .0

Project Description:

Objectives: During previous years we have carried out extensive clinical studies comparing various radiopharmaceuticals for the detection of endocranial tumors and other space occupying lesions (Di Chiro, G., Spar, I. L., Bale, W. F., Laskowski, E. J., Goodland, R. L., and Matthews, W. B.: RIAF (radioiodinated anti-fibrinogen) encephalography. First experiences. Acta Radiol. 1:967-971, 1963; Di Chiro, G., Ashburn, W. L., and Grove, A. S.: Which radioisotopes for brain scanning? Neurology 18:225-236, 1968; Di Chiro, G.: Relative value of air studies, angiography, and scanning in the diagnosis of glial intracranial tumors. In: Neurological Surgery 2:292-317, 1968; and Schall, G. L., Zeiger, L. S., Di Chiro, G., Briner, W. H., and Matsen, F.: Clinical comparison of two ^{99m}Tc tracers for brain scanning: Pertechnetate vs. labeled albumin. Radiology. (In press). These clinical studies have not been conclusive. It is evident that individual lesions are detected by the use of one radioisotope while they are missed when scanning with other radioactive tracers. We have not been able, however, to establish definite and consistent patterns of detecting reliability of the various tracers for the different endocranial lesions. It was for this reason that in the mid 60's we initiated the cooperation with the distinguished virologist Dr. G. F. Rabotti. We intended to develop an experimental brain tumor medel in large animals (dogs) which could be used for radioactive scanning purposes and comparison of the various radiopharmaceuticals for the detection and localization of endocranial and space occupying lesions. We were successful in developing this model (Grove, A. S., Jr., Di Chiro, G., and Rabotti, G. F.: Rous sarcoma virus (RSV) induced brain tumors in dogs with a review of experimental intracranial and neurogenic tumors. J. Neurosurg. 26:465-477, 1967). In addition, many years ago we also developed an experimental model of hemorrhagic lesion in dogs (see ADDENDUM in Di Chiro, G., Spar, I. L., Bale, W. F., Laskowski, E. J., Goodland, R. L., and Matthews, W. B.: RIAF (radioiodinated anti-fibrinogen) encephalography. First experiences. Acta Radiol. 1:967-971, 1963). We have, therefore, available to us, both a model of primary, virus induced canine glial brain tumor, and a model of hemorrhagic cerebral lesions in dogs. A rectilinear scanner, specifically purchased for these experimental studies, is already in full operation. We have proven that we may clearly demonstrate the radioactive brain uptake in dogs. This uptake distributes itself in a constant anatomical pattern and within this pattern, we hope to be able to demonstrate by scanning, brain tumors and bleedings.

Methods Employed: A wide variety of radiopharmaceuticals are (or will be) injected in dogs harboring experimental brain tumors or hemorrhagic cerebral lesions. For the time being we are concentrating our attention on the commonly employed radioisotopes: $^{99m}TcO_4^-$, ^{131}I-HSA, ^{203}Hg-chlormerodrin, ^{197}Hg-chlormerodrin, ^{99m}Tc-HSA. Obviously, however, the choice of the radiopharmaceutical which may be used is unlimited.

Major Findings: At this point we have developed the brain scanning technique in dogs. The cerebral distribution of the radioactive tracers in these animals follows a distinctive anatomical pattern, particularly as seen in the vertex (submento-vertex; axial) projection. The activity in the salivary glands, mouth, nose, and soft tissue surrounding the skull, although very significant, does not in this particular view prohibit a recognition of the relatively low background area of the brain. In this low background area we hope that the tumoral and hemorrhagic lesions will be easily recognizable. Indeed, in one instance, a tumor has been clearly shown by scanning.

Significance to Bio-Medical Research and the Program of the Institute: Scanning of experimental tumors and hemorrhagic lesions represents, in our opinion, the logical and practical model to compare the relative merits of the various radiopharmaceuticals currently used in man for the detection and localization of cerebral neoplasms. The number of radioactive tracers that can be tested by this system is essentially unlimited, and the only problem will be that of selecting "the magic bullet." We are particularly interested in testing radioactivity tagged anti-tumoral drugs. These tests will be helpful in two ways: on one side we should learn if indeed the anti-tumoral drug concentrates in the neoplasm; on the other hand, the "cold"anti-tumoral pharmaceutical could be used to treat an experimental tumor. At a certain stage of the treatment the same "hot" pharmaceutical could be used to grade the response (by scanning) of the neoplastic lesion to the anti-tumoral drug.

Proposed Course of Project: This is a long-term project with practically unlimited possibilities. Actually, the only limitations will be time and personnel. For the rest, the road is open for appraisal of the already in use or the theoretically usable radiopharmaceuticals.

In a selected number of cases, we will carry out scanning tomography with the prototype Tomoscanner which will soon be available to us. By layer tomography we hope to be able to increase the resolution of our scanning methods and, among other goals, establish in an experimental model, the minimum size resolution capabilities of our Tomoscanner system.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 SN/NR 1866(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Cerebral Blood Flow by Radiographic and Radioisotopic Methods.

Previous Serial Number: None

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Leslie S. Libow, M.D.
Steven M. Larson, M.D.
Michael Vermess, M.D.
Sherman C. Stein, M.D.

Cooperating Units: Laboratory of Clinical Science, National Institute of Mental Health, NIH

Diagnostic Radiology Department, Clinical Center,NIH

Department of Nuclear Medicine, Clinical Center, NIH

Man Years:

Total:	.3
Professional:	.3
Others:	.0

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBF), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologically altered conditions is not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, and loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBF. We intend, therefore, to pursue the matter of extracting information regarding the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic observations of the cerebral vessels will represent the main tool to be used in this study.

1) One group of observations deals with the plain skull x-rays of human patients, taking advantage of the fact that, particularly in the aged individuals, certain areas of the cerebral vessels present calcifications of various types. The pattern and degree of this calcification is correlated to the CBF.

2) A second group of observations deals with a study aimed to establish the interhemispheric crossover after bilateral injection in the internal or common carotid arteries of x-rays contrast medium for conventional radiographic angiography, followed by the injection of ^{99m}Tc-pertechnetate for radioisotope angiography. The two angiographic methods--radiographic and radioisotopic--are tested against each other to establish which is more sensitive and to evaluate the degree of interhemispheric communication corresponding to the various anatomical arrangements in the Circle of Willis. Some functional observations will also be made (WADA's test) and attempts to artificially modify this crossover (compression techniques) will be used.

3) Following the research of B. A. Ring and M. M. Waddington we have been interested in the angiographic changes of the small branches of the middle cerebral arteries in focal motor epilepsy. We are particularly concentrating our attention on the operculofrontal branches supplying the premotor area and the artery (or arteries) that are found in the central sulcus. Ring and Waddington have reported a very high degree of positive findings (occluded small branches) in cases of focal motor epilepsy without obvious pathology (tumors, hemangiomas, AV malformations, porencephalic cysts). We have been checking carotid angiograms of our large series of epileptic patients.

Major Findings: 1) Radiographic calcification of the carotid artery siphon is frequently (75.6%) encountered in healthy, elderly males. This calcification may be divided, on the basis of its appearance, in two types: intimal and medial. This subdivision is supported by various radiographic-pathologic investigations. Patients with intimal carotid siphon calcification have diminished CBF values (52.1 ml/100g/min.). Subjects with medial calcification, on the other hand, have CBF values (60.6 ml/100g/min.) closely resembling those found in optimally healthy elderly males (57.9 ml/100g/min.). Thus, the radiographic carotid siphon calcification acquires a new and more selective physiological significance, and potentially a more meaningful clinical value.

2) Until now, eight patients have been studied in the comparative investigation dealing with interhemispheric crossover evaluated by radiographic and radioisotopic angiographic methods. As soon as a larger group of observations will be available, we will start evaluating the data.

3) Our angiographic observations of the small branches of the middle group in cases of focal motor epilepsy without obvious cause, do not confirm the highly optimistic (100%) positive results encountered by Ring and Waddington in a similar group of patients. Preliminary results would indicate that in a few of these cases the arterial supply to the premotor and motor strips is indeed altered, but the percentage of positive findings is by no means as high as in the other investigators' reports.

Significance to Bio-Medical Research and the Program of the Institute: Radiographic observations of normal and pathological cerebral vessels in normal and pathologic conditions still remains a basically important and practical tool of clinical evaluation. Additional work in this area should shed light on some of the controversial subjects connected with the normal and pathologic CBF.

Proposed Course of Project: In clinical material we intend to pursue further our studies of carotid calcification and CBF, the interhemispheric crossover and its modifications, and the possible changes of the small branches of the middle group supplying the premotor and motor strips in cases of focal motor epilepsy.

We are also in the preliminary stage of establishing a conceptually new type of radioisotopic method to evaluate the CBF.

Finally, in the next few months we intend to begin microangiographic studies after experimental occlusion of cerebral arteries (particularly the middle cerebral) in primates. We are planning to observe the changes of the small vessels with special emphasis on acute experiments.

Honors and Awards: None

Publications: Di Chiro, G., and Libow, L. S.: Carotid siphon calcification and cerebral blood flow in the healthy aged male. Radiology (In press).

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Branch of Electroencephalography and Clinical Neurophysiology
Intramural Research
National Institute of Neurological Diseases and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This Branch has provided a clinical diagnostic service for all the patients in the Clinical Center requiring an electroencephalographic examination, either as part of their routine work-up or as part of specific investigative projects originating in other Branches of this Institute or in other Institutes. This form of activity represents a total of man-years about 5.5 (1.20 professional and 4.30 technical-secretarial).

From the time the last report was prepared (April 9, 1970) to that of the present report (April 13, 1971) a total of 1,582 EEG tracings have been obtained and interpreted, following referrals from the various Institutes, and specifically:

INSTITUTE	NO.	%
NINDS	1,114	70.3
NCI	149	9.5
NIMH	102	6.5
NHI	98	6.2
NIAMD	62	3.9
NIAID	46	2.9
NIDR/EHS	11	.9
	1,582	100.0

As in the past the largest number of examinations have been performed for patients admitted by our Institute and, chiefly by its Branch of Surgical Neurology, whereas the total number of referrals from other Institutes has slightly decreased, accounting for less than one-third of total referrals. The above total figure includes about 50 examinations carried out with portable equipment at the patient's bed and/or in intensive care; it also includes 45 studies with chronically implanted electrodes, obtained in 9 patients from the Branch of Surgical Neurology. As a service for, and in cooperation with this same Branch, an additional 25 records were obtained directly from the exposed cerebral cortex in the course of surgical procedures for the treatment of seizures.

2. Research Activity

Of the 14 research projects included in the previous Annual Report, seven were completed and the results have already appeared in the form of publications (4 projects) or are currently in press (3 projects). Two projects (68 EEG/CN 1553 and 70 EEG/CN 1794) have been discontinued due to the departure of the principal investigators and another (70 EEG/CN 1793 not included in this report) is still pending for the same reasons but its completion is expected in the coming fiscal period.

The present report includes 15 projects. Of the four projects which were carried through the period covered by this report, two (EEG/CN 1798 and 1799) should be completed in the near future and, two (EEG/CN 1795 and 1966) are now completed and the results have already appeared in several publications. The latter, together with two new projects (also completed) deal with physiology and pharmacology of the neurosecretory cells in the supraoptic and paraventricular regions of the hypothalamus. Specifically they deal with the response pattern of these elements to stimulation of the neurohypophysis, vagus and carotid sinus nerves and to a number of microiotophoretically applied cholino mimetic agonists and antagonists and other pharmacological agents. Recurrent inhibition could be demonstrated as a result of antidromic stimulation of the axons these cells send to the neurohypophysis, suggesting the existence of mechanisms (negative feed-back loop) controlling their neurosecretory activity. The results of iontophoretic administration of various agents to these same hypothalamic cells suggest that a) norepinephrine is inhibitory and acetylcholine is both inhibitory and excitatory at synapses ending on these cells within the supraoptic region; b) angiotensin and prostaglandin possess an excitatory action on these cells (but have little or no effect on cortical neurons); c) GABA and glycine depress their activity, such an effect being counteracted respectively by picrotoxin and strychnine; d) antidiuretic hormone depresses their activity (but excites that of cortical neurons). Another new project, which investigates the thermosensitivity of hypothalamic cells, is still incomplete. Preliminary results indicate a prominent degree of temperature-dependent activity in a large number of neurosecretory cells. Still pending, but to be completed in the near future is another new project dealing with the basic membrane properties of neurons of Navanax inermis and their changes following the administration of salicylate. The major, preliminary findings of this investigation are summarized in the individual project description (EEG/CN 1871).

The pathophysiological substratum at the basis of epileptiform activity is investigated in another new project utilizing extracellular and intracellular recording techniques and the effects of topical application of penicillin on the cat hippocampus as experimental model. The main objects of this project, which is still incomplete, are a) to determine the role of the various types of neurons in the production of interictal paroxysmal activity and in the transition from this into rhythmical, self-sustained ictal activity, and b) to define the minimal amount of synaptic organization sufficient for the development of these typical epileptiform manifestations. Related to this project is another, recently started, one dealing with an anatomical investigation of the synaptic organization of the preceding model and, specifically with the electron microscopic study of the cat hippocampus. Project EEG/CN 1877, refers to a brief non-experimental contribution. It consists of the description and discussion of various intracellular recording techniques. This project is completed. It will represent the content of a special chapter to be included in a textbook of physiological psychology. Project EEG/CN 1915 was also started recently (in collaboration with the Behavioral Biology Branch of NICHD) and deals with a morphological and physiological investigation of the synaptic organization in spinal cord tissue culture (chick embryo). Preliminary findings suggest the presence of an early differentiation and of synaptic connections between neuronal elements, and their capacity to generate all-or-nothing action potentials.

Three new projects utilize patient material. One is a study of epileptic patients with electrographic evidence of foci localized within the frontal-centro-parietal regions of the brain. The main purpose of this study is to analyze all the various sensory, motor, autonomic and other patterns characterizing the seizure episodes that occur in this group of patients and to determine the relative localizing and lateralizing significance of each of these patterns. This study which is based on the analysis of some 200 cases, should be completed in the near future. Its results should prove of practical usefulness for the topographical identification of the epileptogenic process and, indirectly, for its surgical elimination. Another study, also performed in epileptic patients, represents an attempt to determine possible sub-clinical effects of the inter-ictal paroxysmal discharges characteristic of such patients. Specifically, it consists of an investigation of photically evoked responses and of their modifications when the eliciting stimulus is applied in close temporal relationship with one of the paroxysmal discharges. Due to the special nature of this study, its complex technical problems and the difficulty of finding suitable subjects for the experiment, no definite results are as yet available. The third clinical project is currently carried on in collaboration with the Branch of Neurological Surgery NINDS and deals with the effects of temporal lobectomy on reaction times and with the presence and course of recovery of such a parametric function. Preliminary results would indicate that this recovery does take place, suggesting the possibility of some neural reorganization in the adult human CNS. This project is still in progress and its completion is expected within the coming fiscal year.

3. Programs considered for the near future.

The immediate goal is the completion of those projects which are currently pending. The field of investigation is expected to continue along essentially similar lines throughout the coming fiscal period.

Serial No. NDS(I)-70 EEG/CN 1795(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Antidromic and orthodromic responses in supraoptic neurosecretory cells

Previous Serial Number: Same

Principal Investigator: J. L. Barker, J. W. Crayton and R.A. Nicoll

Other Investigators: None

Cooperating Units: Division of Special Mental Health Research
Laboratory of Neuropharmacology
St. Elizabeth's Hospital, NIMH

Man Years:

Total:	.10
Professional:	.10
Other:	0

Project Description:

Objectives and Methods Employed: Same as last year.

Major findings: Same as last year. In addition: approximately 10% of neurosecretory cells exhibited all-or-none jumps to shorter latencies as the strength of neurohypophysial antidromic stimulation was increased, suggesting branching of neurosecretory axons. Carotid sinus stimulation evoked long latency (83 msec) excitations in antidromically identified supraoptic neurosecretory cells. Neither vagal nor carotid sinus nerve stimulation had any effect on antidromically identified paraventricular neurosecretory cells. Stimulations of the neurohypophysis caused inhibition of both paraventricular and supraoptic neurosecretory cell activity at stimulus strengths below threshold for antidromic activation, indicating that neurosecretory cells are capable of participating in synaptic events. This recurrent inhibition might serve to function as a short, negative feedback loop controlling neurosecretory cell activity.

Significance to Bio-medical Research and the Program of the Institute: Same

Proposed Course of the Project: Completed

Honors and Awards: None

Publications: Nicoll, R. A., Crayton, J. W. and Barker, J. L., Antidromic and orthodromic responses of neurosecretory cells, Physiologist 13: 272, 1970.

Barker, J. L., Crayton, J. W. and Nicoll, R. A., Supraoptic neurosecretory cells: Autonomic modulation, Science 171: 206-207, 1971.

Barker, J. L., Crayton, J. W. and Nicoll, R. A., Antidromic and orthodromic responses of paraventricular and supraoptic neurosecretory cells (in submission to Brain Research).

Serial No. NDS(I)-70 EEG/CN 1796(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Responses of supraoptic neurosecretory cells to microiontophoretically applied substances

Previous Serial Number: Same

Principal Investigator: J. L. Barker, J. W. Crayton and R.A. Nicoll

Other Investigators: None

Cooperating Units: Division of Special Mental Health Research
Laboratory of Neuropharmacology
St. Elizabeth's Hospital, NIMH

Man Years:

Total:	.20
Professional:	.20
Other:	0

Project Description:

Objectives and Methods Employed: See last year.

Major findings: Same as last year. In addition: the inhibitions and depressions following ACh microiontophoresis were further studied by using cholinomimetic agonists and antagonists. Muscarinic agonists (carbachol and acetyl- -methylcholine) always depressed neurosecretory cells. Nicotine excited two-thirds of responsive neurosecretory cells. Atropine, an antimuscarinic agent, blocked both ACh and muscarinic depressions in a majority of cells tested, but failed to block either ACh or nicotine excitations. Dihydro- -erythroidine, an anti-nicotinic agent, blocked both nicotinic and ACh excitations, but failed to antagonize ACh or muscarinic depressions. The results suggest that neurosecretory cells possess two functionally distinct cholinoceptive sites: a nicotinic receptor site whose activition by ACh leads to excitation of neurosecretory cells and a muscarinic receptor site, activation of which causes depression of neurosecretory activity. These findings are consistent with previous pharmacologic research which has shown that nicotine stimulates (and norepinephrine inhibits) the release of ADH. Thus, our results suggest that norepinephrine is inhibitory and ACh both inhibitory (muscarinic) and excitatory (nicotinic) at synapses ending on the supraoptic neurosecretory cell.

Significance to Bio-medical Research and the Program of the Institute: Same

Honors and Awards: None

Publications: Crayton, J. W., Barker, J. L. and Nicoll, R. A. Adrenergic and cholinergic responses in neurosecretory cells. *Pharmacologist* 12: 270, 1970.

Barker, J. L., Crayton, J. W. and Nicoll, R. A. Supraoptic neurosecretory cells: Adrenergic and cholinergic sensitivity, *Science* 171: 208-210, 1971.

Barker, J. L., Crayton, J. W. and Nicoll, R. A., Noradrenaline and Acetylcholine reponses of supraoptic neurosecretory cells. *J. Physiol*. (in press).

Serial No. NDS(I)-70 EEG/CN 1798(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrical correlates of active Na transfer in Aplysia neurons: The effect of metabolic inhibitors (cyanide) on membrane properties

Previous Serial Number: Same

Principal Investigator: D. O. Carpenter and J. L. Barker

Other Investigators: None

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years:

Total: .05
Professional: .05
Other: 0

Project Description:

Objectives: See last year.

Methods employed: Same

Major findings: Same

Significance to Bio-medical Research and Programs of the Institute: Same

Proposed Course of the Project: Project to be completed within the coming fiscal year.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 EEG/CN 1799(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of salicylate on basic membrane properties of _Aplysia californica_ neurons

Previous Serial Number: Same (See also 71 EEG/CN 1871(c))

Principal Investigator: J. L. Barker and H. Levitan

Other Investigators: D. O. Carpenter

Cooperating Units: Behavioral Biology Branch, NICHD
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	.15
Professional:	.15
Other:	0

Project Description:

Objectives and Methods Employed: Same as last year.

Major findings: In a pilot project some 70 neurons in the abdominal ganglion of _Aplysia californica_ were exposed to relatively low (3mM) concentrations of sodium salicylate. It was found that R2 cells always hyperpolarized and R14 cells always depolarized in the presence of salicylate. Other cells either hyperpolarized slightly or did not respond.

The second part of this project is currently underway and consists of a careful evaluation of both the metabolic and ionic processes which determine the membrane potential of identified cells and the effect of salicylate on them. We have done extensive work on R2. This cell hyperpolarizes in response to salicylate but the membrane resistance does not appear to change. The Emlog K curve for this cell is quite complex, depolarizing below $[K^+]_o$ = 10 mM, hyperpolarizing between 10 and 20 mM $[K^+]$, and then following $[K^+]$ so that the average slope over 20 $\leqslant$ $[K^+]_o$ $\leqslant$ 100 mM is about 35 mV/10 fold change $[K^+]_o$. Salicylate hyperpolarizes the curve up to K $\simeq$ 20 mM at which point it increases the Emlog K slope from 35 mV to 58 mV/ 10 fold change $[K^+]$. A variety of chloride substitutes (isethionate, acetate, methylsulfate, proprionate) have been tried but all hyperpolarize the cell, suggesting that the R2 membrane is permeable to these ions. The Emlog K curve in low chloride solutions is generally similar to control though with an increased slope (47 mV average) for $20 \leqslant [K^+]_o \leqslant 100$ mM. Salicylate does not erase the hyperpolarizing effect of chloride replacement, rather it appears to "potentiate" it by making the amplitude of the hyperpolarization bigger. Removal of $(Na)_o$ produces a hyperpolarization of 5-10 mV which is not changed by salicylate. The Emlog K curve in no Na is more linear than in control with the slope for $(K^+) > 10$ mM At $T° < 5°C$ the Emlog K curve is also more linear though its slope at $K > 20$ mM is similar to control values. Salicylate again increases the slope. Ouabain on two occasions has attenuated the hyperpolarization for $10 \leqslant (K^+)_o \leqslant 20$ mM and has reduced the slope for (K^+) values $\geqslant 20$ to ~ 20 mV/10 fold change external K^+; salicylate again increased the slope.

Significance to Bio-medical Research and the Program of the Institute: Same

Proposed Course of the Project: Several more key experiments are planned (chloride transient using $SO_4^=$ as substitute and the effect of salicylate on it; ACh response, its depending on $(Cl)_o$ and the effect of slicylate on it) before this project may be considered completed.

Honors and Awards: None

Publications: None (Preliminary findings presented in a seminar at Yale Department of Environmental Physiology, January 4, 1971.)

Serial No. NDS(I)-71 EEG/CN 1868(c)
1. Electroencephalography and
Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical seizure patterns in patients with suprasylvian (fronto-centro-parietal) epileptogenic foci

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan

Other Investigators: Leo Goldhammer

Cooperating Units: Department of Neurology, George Washington Univ.

Man Years:

Total: .55
Professional: .50
Other: .05

Project Description:

Objectives: To analyze the various sensory, motor, autonomic and other symptoms which characterize the ictal episodes in patients with electroencephalographic evidence of epileptiform activity in the frontal, central and parietal regions.

Methods employed: Data derived from about 200 patients with epileptic seizures selected on the basis of their EEG findings as specified in the objectives. All their records and clinical charts are reviewed and all the observed and described seizures for each patient are analyzed. The pattern of each seizure (up to 25 in any given patients) and/or the various motor, sensory and other components of each seizure are correlated with the EEG, pathological and other clinical findings.

Major findings: On the basis of their EEG findings the patients were subdivided into various groups (prefrontal, frontal-inferior frontal, fronto-central, centro-parietal, parasagittal, etc.). The localizing and lateralizing value of each clinical seizure pattern (or component thereof) are currently in the process of being evaluated. Worth noting among the preliminary findings are a) the relatively high incidence of automatism and autonomic phenomena in cases without obvious involvement of rhinencephalic structures by the epileptogenic process; b) the presence and complexity of motor phenomena and the high incidence of tonic postures in most patients of all groups; c) the occurrence of bilateral symptoms (in the same seizure or in different ictal episodes in the same patients) even in the absence of bilateral EEG involvement; d) the poor lateralizing value of certain tonic-adversive movements.

Significance to Bio-medical Research and Programs of the Institute: This project should provide useful information of diagnostic significance for the localization and lateralization of the epileptogenic process and, eventually, for its surgical elimination.

Proposed Course of the Project: All data have been collected and tabulated. Their analysis should be completed within the next six months.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1869(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The conditioning effect of epileptiform discharges on visually evoked potentials

Previous Serial Number: None

Principal Investigator: M. J. O'Connor

Other Investigators: C. Ajmone Marsan

Cooperating Units: Laboratory of Technical Development of NINDS and NIMH and Branch of Neurological Surgery, NINDS

Man Years:

Total:	.16
Professional:	.10
Other:	.06

Project Description:

Objectives: Investigation of the potential evoked by photic stimulation in epileptic patients and of the effect exerted upon this waveform by a co-existing epileptiform discharge or by one preceeding the photic stimulus at different intervals.

Methods employed: This study is carried out in epileptic patients with chronically implanted electrodes. The input from the electrode(s) in the most active area(s) (i.e., those showing the highest incidence of epileptiform activity of the largest amplitude and shortest duration is amplified and fed into a digital trigger circuit which, upon recognition of a waveform possessing the appropriate polarity, amplitude, rise time and total duration, triggers a light flash at variable, predetermined delays. The EEG over the occipital areas is monitored on a CRO and, together with the activity being fed into the triggering circuit and the trigger pulse, is recorded on magnetic tape. The evoked potential is analyzed on a PDP12. Control experiments, which are performed simultaneously by appropriate digital coding include the recording of photically evoked potentials in the absence of epileptiform activity and of the wave forms accompanying epileptiform activity in the absence of a flash.

Major Findings: Thus far, the number of experiments performed and analyzed is insufficient to draw any conclusion.

Significance to Bio-medical Research and the Program of the Institute: This project should provide some information on the possible effect on sensory mechanisms of sub-clinical inter-ictal abnormal discharges which characterize the EEG of epileptic patients.

Proposed Course of the Project: Experiments to be performed in all suitable subjects whenever available. The project should be carried out and completed within the coming fiscal year.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1870(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Reaction time patterns in patients before and after temporal lobectomy

Previous Serial Number: None

Principal Investigator: C. J. Herman

Other Investigators: P. Fedio

Cooperating Units: Surgical Neurology Branch, IR, NINDS

Man Years:

Total: .10
Professional: .10 (J.H.)
Other: 0

Project Description:

Objectives: To define the time course of recovery of reaction times of ipsi- and contralateral hands following temporal lobectomy.

Methods employed: Reaction time of each hand to free field light and tone are measured before and after operation in patients treated for focal epilepsy by temporal lobectomy. Measurement of reaction times is performed twice before operation and every 3-4 days after operation.

Major findings: Previous studies indicate that the time course of improvement in patient's reaction time following unilateral ischemic stroke can be accounted for only in part by changes in blood flow, edema and its resolution, partial demyelination and remyelination, surround glial reaction and neuronal involvement in scar formation. Preliminary results of the present study indicate that a similar time course of recovery is seen with the more defined, cleaner unilateral lesion of lobectomy. This may indicate the occurrence of neural reorganization, either anatomical or physiological or both, in adult human central nervous system following limited damage.

Significance to Bio-medical Research and the Program of the Institute: This project is of importance due to its close relationship to one of the major activities of the Branch of Surgical Neurology.

Proposed Course of the Project: Continued reaction time testing of a sufficiently large number of patients undergoing temporal lobectomy together with review of neuropathological data on type and amount of tissue removed as well as the course of healing of the temporal lobe stump is expected to provide support for or rejection of the hypothesis that neural reorganization at a functional level occurs in human CNS.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1871(c)
1. Electroencephalography and
Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of salicylate on basic membrane properties of *Navanax inermis* neurons

Previous Serial Number: None (See also NDS(I)-70 EEG/CN 1799(c))

Principal Investigator: J. L. Barker and H. Levitan

Other Investigators: None

Cooperating Units: Behavioral Biology Branch, NICHD
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	.15
Professional:	.15
Other:	0

Project Description:

Objectives and Methods Employed: Similar to those of NDS(I)-70 EEG/CN 1799(c).

Major findings: Identified Navanax neurons have a greater resting potential than R2 *Aplysia* cells, do not show the anomalous rectification properties of *Aplysia* cells, and possess little resting Na conductance (unlike *Aplysia*). The Emlog K curve is simpler than Aplysia's R2, showing a 2-8 mV change/10 fold $(K^+)_o$ change for $1 \leqslant (K^+)_o \leqslant 10$ mM and an average slope of 33 mV for $10 \leqslant (K^+)_o \leqslant 100$ mM. Salicylate increased the RMP and decreased the R_m of all cells studied in a reversible, dose-dependent manner. Synaptic activity was greatly attenuated. Salicylate increased the Emlog K curve slope from 33 to 58 mV/10 fold change (K^+). The salicylate Emlog K curves crossed the control curve at about the equilibrium potential for chloride. Chloride transients were studied using acetate, proprionate, isethionate, methylsulfate, sulfate and bromide replacements for $(Cl^-)_o$. The amplitude and direction of the transients depends on the particular substitute used as well as the $(K^+)_o$. Salicylate completely attenuated these transients. The slope of the Emlog K curve in low $(Cl^-)_o$ was 46 mV. Removal of external Na caused

1-5 mm hyperpolarizing transients which were not altered by salicylate. The Emlog K curve slope in NoNa was about 40 mV. Salicylate decreased the drop in R_m during the chloride component of the ACh response but did not alter the R_m during the Na component. Neither cold nor ouabain affected the salicylate increase of the Emlog K curve slope.

Significance to Bio-medical Research and Programs of the Institute: These results provide the ionic mechanisms underlying the action of salicylate on molluscan neurons. Salicylate reduces the input to and the output from a particular cell by increasing its potassium permeability and decreasing its chloride permeability. These findings should provide the basis for investigating the effects of salicylate on vertebrate neurons.

Proposed Course of the Project: This project will shortly be completed.

Honors and Awards: None

Publications: Barker, J. L. and Levitan, H., Effects of salicylate on membrane permeability of molluscan neurons. Science (in press).

Barker, J. L., Levitan, H. and Carpenter, D. O., Effects of salicylate on molluscan neurons. Fed. Proc. (in press).

Serial No. NDS(I)-71 EEG/CN 1872(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The responses of supraoptic neurosecretory cells to angiotensin and prostaglandin

Previous Serial Number: None

Principal Investigator: J. L. Barker and R. A. Nicoll

Other Investigators: None

Cooperating Units: Division of Special Mental Health Research
Laboratory of Neuropharmacology, NIMH

Man Years:

Total:	.10
Professional:	.10
Other:	0

Project Description:

Objectives: Both angiotensin and prostaglandin have been reported to release antidiuretic hormone (ADH) following intracarotid injection. Furthermore, intrahypothalamic application of angiotensin (by cannula) also results in a release of ADH. These findings prompted us to examine the responsiveness of supraoptic neurosecretory cells to the microiontophoretic application of these substances.

Methods employed: Cats were anesthetized with sodium pentobarbital, tracheotomized and placed supine in a stereotactic apparatus. A buccal approach was used to expose both the pituitary gland and the optic chiasm. Angiotensin, prostaglandin and norepinephrine were microiontophoresed onto antidromically identified supraoptic neurosecretory cells. In addition, angiotensin and prostaglandin were administered intravenously. Angiotensin was also applied microiontophoretically to cortical neurons in order to provide a control profile of response to this polypeptide.

Major findings: Both angiotensin and prostaglandin excited a majority of the supraoptic neurosecretory cells tested. When applied to the cortex, angiotensin had little, if any, effect.

Prostaglandin blocked the norepinephrine depressions in a small percentage of unidentified neurons in the supraoptic region and antagonized a minority of the norepinephrine depressions of neurosecretory cells. Intracarotid injection of these substances in doses which produced significant cardiovascular change caused little, if any change in the activity of these cells.

Significance to Bio-medical Research and the Program of the Institute: These results suggest that the polypetide angiotensin, which governs the release of the salt-retaining hormone aldosterone also appears to stimulate the (coincident) release of ADH, the water retaining hormone. This would provide a mechanism whereby both salt and water retentions would be activated in concert. The effect appears to be fairly specific since microiontophoresis of angiotensin onto cortical neurons was without effect. Prostaglandin has also been shown to excite supraoptic neurosecretory cells, thus providing evidence of the neural correlate to the endocrine response of the nucleus. The fact that we could not block a majority of norepinephrine depressions of neurosecretory cell activity which prostaglandin suggests that the mechanism of excitation is not through this route.

Proposed Course of the Project: Completed.

Honors and Awards: None

Publications: A short communication is being written.

Serial No. NDS(I)-71 EEG/CN 1873(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pharmacology of recurrent inhibition in the supraoptic neurosecretory system

Previous Serial Number: None

Principal Investigator: J. L. Barker and R. A. Nicoll

Other Investigators: None

Cooperating Units: Division of Special Mental Health Research
Laboratory of Neuropharmacology
St. Elizabeth's Hospital, NIMH

Man Years:

Total:	.20
Professional:	.20
Other:	0

Project Description:

Objectives: The recent demonstration of "recurrent inhibition" in neurosecretory systems has prompted us to study the pharmacology of this inhibition. We applied pharmacologic agonists and antagonists both microiontophoretically and intravenously in an attempt to identify the inhibitory transmitter in this pathway.

Methods employed: Cats were anesthetized with sodium pentobarbital, tracheotomized and placed supine in the sterotactic apparatus. A buccal approach was used to expose both the pituitary gland and optic chiasm. GABA, glycine, antidiuretic hormone (ADH), picrotoxin, bicuculline, strychnine, and d, l, homocysteic acid (DLH) were applied by means of iontophoresis onto antidromically identified supraoptic neurosecretory cells. Atropine, bicuculline and strychnine were also given intravenously (to flaxedilized, artificially respired cats) after control post-stimulus histograms of recurrent inhibition. ADH was microiontophoresed onto cortical neurons as a control for responses observed in supraoptic neurosecretory cells.

Major findings: GABA and glycine depressed the activity of all spontaneously active (and DLH-induced supraoptic neurosecretory cells. GABA depressions were readily blocked with either bicuculline or picrotoxin. Glycine depressions were blocked with strychnine. Iontophoresis of these blocking agents (and atropine) did not alter the post-stimulus histogram inhibitory profile. Similarly, intravenous administration of these agents (in convulsing doses) did not reduce the inhibition seen on post-stimulus histogram. ADH depresssed 80% of responsive supraoptic neurosecretory cells and excited nearly 90% responsive cortical neurons.

Significance to Bio-medical Research and the Program of the Institute: Our results suggest that GABA, glycine and acetylcholine (muscarinic component) are not likely to be the transmitter substance mediating this inhibitory pathway. The fact that a good correlation was found between those cells exhibiting recurrent inhibition and those depressed by ADH supports the idea that ADH may serve as a transmitter in this pathway. It is thus possible that neurosecretory cells elaborate ADH both for "hormonal" and "transmitter" functions.

Proposed Course of the Project: Completed.

Honors and Awards: None

Publications: Nicoll, R. A. and Barker, J.L. The Pharmacology of Recurrent Inhibition in the Supraoptic Neurosecretory System (in submission to Brain Research).

Serial No. NDS(I)-71 EEG/CN 1874(c)
1. Electroencephalography and
Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Thermosensitivity of supraoptic neurosecretory cells

Previous Serial Number: None

Principal Investigator: J. L. Barker and R. A. Nicoll

Other Investigators: None

Cooperating Units: Division of Special Mental Health Research
Laboratory of Neuropharmacology
St. Elizabeth's Hospital, NIMH

Man Years:

Total:	.05
Professional:	.05
Other:	0

Project Description:

Objectives: Since a direct correlation between the temperature of the hypothalamus and the release of antidiuretic hormone has been reported, we chose to investigate the neural correlates of this endocrine behavior by varying the temperature of supraoptic neurosecretory cells and recording the resultant extracellular activity.

Methods employed: Cats were anesthetized with sodium pentobarbital, tracheotomized and placed supine in the stereotactic apparatus. A buccal approach was used to expose the posterior pituitary and optic chiasm. NaCl-filled glass micropipettes were employed to record the activity of antidromically-identified supraoptic neurosecretory cells. The temperature of the hypothalamus (an brain) was varied 4° to 8° centigrade by perfusing cold or hot alcohol through metal coils placed at the carotid sites.

Major findings: Nearly all supraoptic neurosecretory cells studied thus far have shown a high degree of temperature dependent activity, suggesting that these cells are inherently and highly temperature sensitive.

Significance to Bio-medical Research and the Program of the Institute: This project is a complement to project 70-1797 of the previous year (completed) and is part of our general interest on the effects of temperature and antipyretics on CNS cells.

Proposed Course of the Project: More research needs to be done before a short communication can be written.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1875a(c)
1. Electroencephalography and
Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cellular Analysis of Penicillin Epileptic Focus

Previous Serial Number: None

Principal Investigator: M. Dichter and C. Herman

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.25
Professional:	.75
Other:	.50

Project Description:

Objectives: To discover the role of various cell types in the production of the epileptiform interictal spike discharge and in the transition from it to long lasting seizure activity. To elucidate the mechanism of action of some anti-convulsants.

Methods employed: Epileptic foci are established in a simplified form of cortex - cat hippocampus. They are studied with extracellular and intracellular microelectrode recording techniques. Methods are being developed to use vital dye filled electrodes for accurate marking of recording sites and individual neurons.

Major findings: During interictal penicillin discharges recorded with gross electrodes, some units fire short bursts of action potentials while the activity of other units is inhibited. These units have been identified as pyramidal cells, the specific firing pattern being correlated with position of the cells relative to the site of penicillin application. In addition, some cells fire long trains of action potentials which long outlast the interictal discharges. Some of these, at least, are also pyramidal cells. Others have been recorded predominantly in slightly deeper layers. Of the latter cell type, there are two subgroups in relation to immediate pre-seizure activity. One subgroup fires more and more intensely as the seizure develops. The second subgroup exhibits a dropping out of firing immediately before a seizure.

Significance to Bio-medical Research and the Program of the Institute: The definition of the basic cellular mechanism of seizure disorder is one of the main interests of the Branch and is an important aspect of the study and treatment of epilepsy, a major goal of the Institute.

Proposed Course of the Project: Continue extra and intracellular recordings to attempt to elucidate those mechanisms underlying the transition from interictal spiking to full seizure activity. Investigate action of diphenylhydantoin on this same preparation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1875b(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Unit analysis of minimal synaptic organization required for penicillin epileptic focus.

Previous Serial Number: None

Principal Investigator: C. Herman and M. Dichter

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.25
Professional:	.75
Other:	.50

Project Description:

Objectives: To define the minimal synaptic organization in the cat hippocampus capable of supporting interictal and ictal manifestations of the penicillin epileptic focus.

Methods employed: Extracellular recording of single neuronal activity is used as an indication of cell activity. A penicillin epileptic focus is established in the cat hippocampus and the effects on unit activity of acute surgical disconnection of various fields of the hippocampus from the remainder of the hippocampus are evaluated. Dye - filled electrodes allow marking and subsequent anatomical localization of neurons with defined firing patterns.

Major findings: Four general patterns of unit activity have been observed in the penicillin epileptic focus in the intact hippocampus. Cells which are spontaneously active are seen during paroxysmal interictal discharges to 1) be completely inhibited (Pattern I) or 2) fire a brief high - frequency burst of spikes followed by inhibition of firing (Pattern II). Other cells have been found which fire a prolonged high frequency burst of spikes during and following the paroxysmal discharge. As the paroxysmal discharges become more frequent and a seizure ensues, these cells show either 1) prolongation and increased frequency of spike trains (Pattern III) or 2) shortening and decreased frequency of spike trains (Pattern IV).

When the CA2-4, dentate gyrus region of a penicillin - treated hippocampus is surgically disconnected from the CA1-2 region, these same four firing patterns can be seen in the CA3-4 and dentate gyrus cells. CA1-2 cell firing patterns after acute disconnections are not yet clearly established.

Significance to Bio-medical Research and the Program of the Institute: The definition of the basic cellular mechanisms of seizure disorder is one of the main interests of the Branch and is an important aspect of the study and treatment of epilepsy, a major goal of the Institute.

Proposed Course of the Project: To further define the localization of the penicillin focus and thus define the nature of "driven" vs. "driving" cells in this focus. Further investigation of the "driving" cells in the antomically restricted and defined focus will then be possible.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1876(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron Microscopic Study of the Cat Hippocampus

Previous Serial Number: None

Principal Investigator: M. Dichter and J. Mendell

Other Investigators: None

Cooperating Units: Medical Neurology, NINDS

Man Years:

Total:	.15
Professional:	.15 (M.D.)
Other:	0

Project Description:

Objectives: Electron microscopy of the cat hippocampus is being performed in an effort to identify the locus of synapses on pyramidal cells. Classical anatomists (Cajal, Lorente de No) have identified an area of pyramidal cells (CA4) without basket cell synapses around their cell bodies. Previous neurophysiological investigation of the hippocampus has suggested that inhibitory synapses occur on the cell bodies by way of axosomatic synapses. This argument has also been extended to other parts of the nervous system and is being considered as a general rule. It has now been shown that the cells of the CA4 region of the hippocampus have recurrent inhibition similar or identical to that seen elsewhere in the hippocampus. Electron microscopy of the CA4 region will reveal the presence or absence of axosomatic synapses in CA4.

Methods employed: Light and electron microscopic examination of glutaraldehyde fixed brain. Seriel micrographs are taken to reconstruct the architecture of individual cells.

Major findings: CA4 pyramidal cell somata are devoid of synapses. CA1 pyramidal cell somata have approximately 30% of their surface covered by axosomatic synapses.

Significance to Bio-medical Research and the Program of the Institute: Understanding synaptic organization of cortex is important for understanding mechanism of maintenance and spread of seizures. This is especially true for inhibitory and recurrent systems.

Proposed Course of the Project: Continue investigation to confirm initial findings. Investigate areas of tightly packed pyramidal cells for tight junctions.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1877(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Intracellular Single Unit Recording

Previous Serial Number: None

Principal Investigator: M. Dichter

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.06
Professional:	.05
Other:	.01

Project Description:

Objectives, etc.: Description and discussion of intracellular recording techniques. To form the object of a chapter for a textbook in physiological psychology, providing a simplified and readable account of how to approach the problem of intracellular recording for an advanced investigator with no prior experience in this field. It should be applicable to those interested in commencing work in this area and those interested in obtaining a conversant knowledge of the techniques and problems of intracellular recording. This chapter will not be sufficient in itself to provide an investigator with all the information needed to begin work on his own.

This chapter covers the technical aspects of intracellular recording including properties of microelectrodes, micromanipulators, problems of movement control, and recording equipment. In addition criteria for good intracellular recording and microelectrode techniques for more specialized usage are discussed.

Significance to Bio-medical Research and the Program of the Institute: This Branch of NINDS has been involved with experiments requiring intracellular recording from nerve cells for many years. This chapter will be an education service to young investigators entering the field of neurophysiology or experimental psychology.

Proposed Course of the Project: Completed - The book should be published within the next year.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 EEG/CN 1915(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Synaptic Organization of Dissociated Spinal Cord Tissue Culture

Previous Serial Number: None

Principal Investigator: M. Dichter and G. Fischbach

Other Investigators: None

Cooperating Units: Behavioral Biology Branch, NICHD

Man Years:

Total:	.20
Professional:	.20 (M.D.)
Other:	0

Project Description:

Objectives: Dissociated chick embryo spinal cord neurons differentiate and form synaptic connections in tissue culture. This is a useful preparation for studying the physiology and pharmacology of synapses in various parts of the nervous system. In spinal cord cultures it is of interest to study the development of synaptic connections, transmitter agents, receptor specializations, and the specificity of the connections.

Methods employed: Chick embryo spinal cord is dissociated with trypsin and plated in petri dishes. At suitable points in development intracellular recordings are obtained from neural elements in the tissue culture. Various combinations of plating density, media, etc. are explored for optinum culture.

Major findings: Neurons do differentiate morphologically and physiologically. Membrane resistance is comparable to values seen in vivo in older animals. The cells can generate all-or-nothing action potentials. Evidence of synaptic connections has been obtained.

Significance to Bio-medical Research and the Program of the Institute: This project should provide useful information on basic neurophysiological phenomena.

Proposed Course of the Project: Continue investigation outlined in "Objectives".

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Neuropathology and Neuroanatomical Sciences, IR
National Institute of Neurological Diseases and Stroke

Igor Klatzo, Chief

Introduction

The most important event for the Laboratory was the activation of two new sections, namely, the Section on Cellular Neurochemistry and the Section on Neurocytobiology. This completes the original plan which envisioned a laboratory consisting of sectional units capable to cover comprehensively main technical approaches used in modern neurobiology. Thus, within the present organization we should be able to correlate morphological observations, from both light- and electron microscopy, with cytochemical, biochemical and physiological data derived from experiments in vivo or from growing nervous tissue in culture.

In line with basically dichotomous structure of our Laboratory, the investigations were directed to elucidation of both normal and abnormal nervous system relationships.

In order to evaluate properly pathological changes induced in experiments, as well as, those observed in clinical neuropathology further studies were carried out on specificity of nervous tissue changes, i.e., recognition of non-specific or artifactitious changes. Various tissue fixation procedures were devised and compared both in normal control animals and in those subjected to various lesions such as axotomy, irradiation, and interruption of cerebral circulation. The principal finding of these studies were reported at The Sixth International Congress of Neuropathology in Paris.

An important but enigmatic structure - area postrema was studied morphologically on a comparative basis. Differences in topography of the floor of fourth ventricle and of area postrema between most commonly used experimental animals, monkey and cat, were described. It is planned to extend these studies to animals suffering from hydrocephalus as this may bring some clues concerning relationship between area postrema and physiology of the cerebrospinal fluid.

Structural basis for synaptic transmission was studied both in brain and in neuromuscular junctions. In the brain, three dimensional reconstructions based on electromicroscopical examination of serial sections were correlated with the light and E.M. observations derived from Golgi impregnated brains. This work has demonstrated reciprocal synapses between dendrites of mitral (multipolar) and granule (axonless) neurons in the olfactory bulb. The reciprocal synapses were excitatory in a mitral-to-granule direction - and inhibitory in granule - to mitral direction. These studies on structure and organization of synapses are to be supplemented by observations on transsynaptic degenerative changes in synaptic endings after removing the excitatory synaptic input to the cell bodies giving rise to these synaptic endings.

Studies on structural changes which accompany physiological changes at nerve-muscle synapses were carried out on frog sartorius nerve-muscle preparations exposed to altered ionic environments or stimulated with various programs. After reaching a desired functional state, the preparations are immediately fixed and prepared for examination with electron microscope. It was shown that with intense electrical stimulation and transmitter release approaching exhaustion the intracellular organelles of the presynaptic terminal undergo well-defined, reversible changes. These included mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus like bodies, apparently as intermediate stages in the recycling of membrane back into synaptic vesicles.

Studies on peripheral nerve and myelin were undertaken in direct observations on living tadpoles and with electron microscope after appropriate fixation. Living peripheral nerves in tails of the tadpoles mounted in specially designed chamber were observed by differential interference microscopy (Nomarski system). Motile, thread-shaped structures appeared to be axonal mitochondria. Myelin internodes were smooth except for occasional variations in contour. The appearance of axons and their myelin sheaths remained essentially unchanged during the early stages of fixation for electron microscopy. It is intended to use this model system in elucidating cellular mechanisms of disease in myelinated nerve fibers. In studies on fetal and newborn rat sciatic nerves, the geometry of Schwann cells and their surface relationship with axons before the onset of myelination was demonstrated to proceed in a defined, orderly fashion. It is kept in mind that abnormalities in this pattern may be important factors in hereditary and developmental neuropathies and the data collected in this study should be useful in interpreting many of the electron microscopic abnormalities that have been reported in human diseases.

Trophic functions of peripheral nervous system were investigated in two ways. One of the aspects concerned the mechanisms by which the nerve regulates the metabolism of the muscle fiber. In these studies denervation produced enzymatic changes which indicated that the muscle fiber is a dynamic cell, capable of undergoing remarkable alterations in its phenotypic appearance in response to a variety of environmental influences. Major among these is the effect of denervation, cross-reinnervation and exercise.

The other aspect studied concerned the problems of neuronal transplantation. The first question here was whether transplanted gustatory neurons would survive, regenerate their nerve fibers, and retain the trophic property of inducing taste bud formation. Autologous and homologous transplantation was performed so that the role of the immune system regarding neuron survival and function could be evaluated. The second question concerned the preservation of functional specificity of neurons in transplantation. The results of these studies revealed that some neurons in the autologous and homologous transplanted nodose ganglion survived, grew out their nerve fibers, and caused taste bud regeneration. This occurred even when a cellular immnue response was present around the homologously transplanted tissue. The observations on denervated rat skeletal sternomastoid muscle into which motor, sensory or autonomic nerves were implanted demonstrated that only motor neurons can functionally innervate muscle. Further elucidation of basic problems of nervous tissue regeneration, transplantation and trophic function is being undertaken in another series of similar experiments.

Studies on permeability of various cellular barriers within the central nervous system (CNS) were conducted in several directions. Horseradish peroxidase (M.W. 40,000) and newly developed microperoxidase (M.W. 1,800) were primarily used as the electronmicroscopically demonstrable tracers, according to cytochemical method of Karnovsky. Introduction of these tracers into various CNS compartments revealed some basic permeability relationships which are of potential significance for interpretation of certain pathological conditions. Thus, it was shown that there is a blood-nerve barrier (BNB), similarly to the blood-brain barrier (BBB). It was established that the morphological basis for the BNB is provided by the tight interendothelial junctions within the endoneurial blood vessels and by the tight junctions joining inner layers of the perineurial sheath. This creates an endoneurial compartment which allows the peripheral nerve to maintain its homeostatic, special chemical environment, similarly as in the brain. In a study of morphological confines of the CSF compartment, peroxidase-impermeable tight junction barriers were described in the outer border of the arachnoid and in the ependymal covering of some special periventricular areas such as median eminence and area postrema.

Injecting microperoxidase directly into a crayfish giant axon, it was possible to demonstrate the passage of the tracer through the gap junction into adjacent axon. This movement occurs only under conditions where the axons are electrically coupled. At the gap junctions between coupled and injected axons, bridges 10-15 Å in diameter cross the gap. These bridges may localize the channels through which microperoxidase crosses from axon to axon. These observations are of basic relevance to how exchanges of substances from cell to cell occur and to pathomechanisms of neoplastic transformation, especially since changes in coupling appear to be a component of cancerous changes. Otherwise, in a separate study, application of peroxidase in experimental brain tumors (induced by viruses) demonstrated easy penetration of the tracer through the fenestrated capillaries and vigorous pinocytotic uptake of peroxidase by the neoplastic cells.

Although the tight junctions between the endothelial cells appear to constitute the morphological basis for the BBB throughout the vertebrate species, one notable exception was found with regard to Elasmobranch fishes. The cerebral capillaries of shark are devoid of tight junctions and the peroxidase tracer passes freely through endothelium but does not spread between the cellular elements of neuropil, as it is the case in other vertebrate species. This may account for a striking resistance of shark to development of brain edema following severe cerebral trauma. Otherwise, studies on effects of ionizing radiation on shark brain revealed again a remarkable resistance of these animals to injury. Even 30,000 rad dose failed to produce any recognizable pathological changes in the shark brain when the brains were studied one year after the exposure.

To approach more directly the basic phenomena of the BBB, a model of osmotic BBB injury was developed. It was possible to standardize injury and to establish thresholds for BBB breakdown at certain osmolalities. The BBB damage of this type is quickly reversible and presumably it is based on opening of tight junctions due to osmotic shrinkage of endothelial cells. Applying double tracer method of Oldendorf (H^3 water and C^{14} test substance), we are now in the process of ascertaining selective features of BBB vulnerability. This data, hopefully, may prove to be useful for assessment of the size of gaps produced by osmotic shrinkage of endothelium, as well as for getting insight with regard to routes of transcapillary passage or transport of various substances including essential nutrients such as glucose and some amino acids.

The newly established Section on Cellular Neurochemistry is still in the early stages of activation. The undertaken investigations are concerned with allosteric control of fructose 1, 6 diphosphate, brain glycogen synthetase and kinetics of brain glycogen. This data is expected to be valuable for interpretation of abnormal accumulation of glycogen in various brain pathological conditions, described in our previous investigations.

Similarly the Section on Neurocytobiology is in initial phase of development. Currently studied are chemical changes in brain of experimentally induced microcephaly. Also, this Section is significantly involved in the mentioned investigations on osmotic BBB injury providing essential to this project radioisotopic assays.

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Neuropathology and Neuroanatomical Sciences, IR
National Institute of Neurological Diseases and Stroke

Igor Klatzo, Chief

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Structural basis for synaptic transmission was studied both in brain and in neuromuscular junctions. In the brain, three dimensional reconstructions based on electromicroscopical examination of serial sections were correlated with the light and E.M. observations derived from Golgi impregnated brains. This work has demonstrated reciprocal synapses between dendrites of mitral (multipolar) and granule (axonless) neurons in the olfactory bulb. The reciprocal synapses were excitatory in a mitral-to-granule direction - and inhibitory in granule - to mitral direction. These studies on structure and organization of synapses are to be supplemented by observations on transsynaptic degenerative changes in synaptic endings after removing the excitatory synaptic input to the cell bodies giving rise to these synaptic endings.

Studies on structural changes which accompany physiological changes at nerve-muscle synapses were carried out on frog sartorius nerve-muscle preparations exposed to altered ionic environments or stimulated with various programs. After reaching a desired functional state, the preparations are immediately fixed and prepared for examination with electron microscope. It was shown that with intense electrical stimulation and transmitter release approaching exhaustion the intracellular organelles of the presynaptic terminal undergo well-defined, reversible changes. These included mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus like bodies, apparently as intermediate stages in the recycling of membrane back into synaptic vesicles.

Studies on peripheral nerve and myelin were undertaken in direct observations on living tadpoles and with electron microscope after appropriate fixation. Living peripheral nerves in tails of the tadpoles mounted in specially designed chamber were observed by differential interference microscopy (Nomarski system). Motile, thread-shaped structures appeared to be axonal mitochondria. Myelin internodes were smooth except for occasional variations in contour. The appearance of axons and their myelin sheaths remained essentially unchanged during the early stages of fixation for electron microscopy. It is intended to use this model system in elucidating cellular mechanisms of disease in myelinated nerve fibers. In studies on fetal and newborn rat sciatic nerves, the geometry of Schwann cells and their surface relationship with axons before the onset of myelination was demonstrated to proceed in a defined, orderly fashion. It is kept in mind that abnormalities in this pattern may be important factors in hereditary and developmental neuropathies and the data collected in this study should be useful in interpreting many of the electron microscopic abnormalities that have been reported in human diseases.

Trophic functions of peripheral nervous system were investigated in two ways. One of the aspects concerned the mechanisms by which the nerve regulates the metabolism of the muscle fiber. In these studies denervation produced enzymatic changes which indicated that the muscle fiber is a dynamic cell, capable of undergoing remarkable alterations in its phenotypic appearance in response to a variety of environmental influences. Major among these is the effect of denervation, cross-reinnervation and exercise.

The other aspect studied concerned the problems of neuronal transplantation. The first question here was whether transplanted gustatory neurons would survive, regenerate their nerve fibers, and retain the trophic property of inducing taste bud formation. Autologous and homologous transplantation was performed so that the role of the immune system regarding neuron survival and function could be evaluated. The second question concerned the preservation of functional specificity of neurons in transplantation. The results of these studies revealed that some neurons in the autologous and homologous transplanted nodose ganglion survived, grew out their nerve fibers, and caused taste bud regeneration. This occurred even when a cellular immnue response was present around the homologously transplanted tissue. The observations on denervated rat skeletal sternomastoid muscle into which motor, sensory or autonomic nerves were implanted demonstrated that only motor neurons can functionally innervate muscle. Further elucidation of basic problems of nervous tissue regeneration, transplantation and trophic function is being undertaken in another series of similar experiments.

Studies on permeability of various cellular barriers within the central nervous system (CNS) were conducted in several directions. Horseradish peroxidase (M.W. 40,000) and newly developed microperoxidase (M.W. 1,800) were primarily used as the electronmicroscopically demonstrable tracers, according to cytochemical method of Karnovsky. Introduction of these tracers into various CNS compartments revealed some basic permeability relationships which are of potential significance for interpretation of certain pathological conditions. Thus, it was shown that there is a blood-nerve barrier (BNB), similarly to the blood-brain barrier (BBB). It was established that the morphological basis for the BNB is provided by the tight interendothelial junctions within the endoneurial blood vessels and by the tight junctions joining inner layers of the perineurial sheath. This creates an endoneurial compartment which allows the peripheral nerve to maintain its homeostatic, special chemical environment, similarly as in the brain. In a study of morphological confines of the CSF compartment, peroxidase-impermeable tight junction barriers were described in the outer border of the arachnoid and in the ependymal covering of some special periventricular areas such as median eminence and area postrema.

Injecting microperoxidase directly into a crayfish giant axon, it was possible to demonstrate the passage of the tracer through the gap junction into adjacent axon. This movement occurs only under conditions where the axons are electrically coupled. At the gap junctions between coupled and injected axons, bridges 10-15 Å in diameter cross the gap. These bridges may localize the channels through which microperoxidase crosses from axon to axon. These observations are of basic relevance to how exchanges of substances from cell to cell occur and to pathomechanisms of neoplastic transformation, especially since changes in coupling appear to be a component of cancerous changes. Otherwise, in a separate study, application of peroxidase in experimental brain tumors (induced by viruses) demonstrated easy penetration of the tracer through the fenestrated capillaries and vigorous pinocytotic uptake of peroxidase by the neoplastic cells.

Although the tight junctions between the endothelial cells appear to constitute the morphological basis for the BBB throughout the vertebrate species, one notable exception was found with regard to Elasmobranch fishes. The cerebral capillaries of shark are devoid of tight junctions and the peroxidase tracer passes freely through endothelium but does not spread between the cellular elements of neuropil, as it is the case in other vertebrate species. This may account for a striking resistance of shark to development of brain edema following severe cerebral trauma. Otherwise, studies on effects of ionizing radiation on shark brain revealed again a remarkable resistance of these animals to injury. Even 30,000 rad dose failed to produce any recognizable pathological changes in the shark brain when the brains were studied one year after the exposure.

To approach more directly the basic phenomena of the BBB, a model of osmotic BBB injury was developed. It was possible to standardize injury and to establish thresholds for BBB breakdown at certain osmolalities. The BBB damage of this type is quickly reversible and presumably it is based on opening of tight junctions due to osmotic shrinkage of endothelial cells. Applying double tracer method of Oldendorf (H^3 water and C^{14} test substance), we are now in the process of ascertaining selective features of BBB vulnerability. This data, hopefully, may prove to be useful for assessment of the size of gaps produced by osmotic shrinkage of endothelium, as well as for getting insight with regard to routes of transcapillary passage or transport of various substances including essential nutrients such as glucose and some amino acids.

The newly established Section on Cellular Neurochemistry is still in the early stages of activation. The undertaken investigations are concerned with allosteric control of fructose 1, 6 diphosphate, brain glycogen synthetase and kinetics of brain glycogen. This data is expected to be valuable for interpretation of abnormal accumulation of glycogen in various brain pathological conditions, described in our previous investigations.

Similarly the Section on Neurocytobiology is in initial phase of development. Currently studied are chemical changes in brain of experimentally induced microcephaly. Also, this Section is significantly involved in the mentioned investigations on osmotic BBB injury providing essential to this project radioisotopic assays.

Serial No. NDS(I)-71 LNNS/CNC 1883
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Allosteric control of fructose-1,6-diphosphatase

Previous Serial Number: NONE

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1.1
Professional: 0.3
Other: 0.8

Project Description:

Objectives: To investigate the allosteric properties of fructose-1,6-diphosphatase in red and white muscle and brain.

Methods Employed: Crude muscle or brain homogenates are analyzed for fructose-1,6-diphosphatase activity by a rapid fluorometric procedure, employing auxiliary enzymes coupled to a pyridine nucleotide system.

Major Findings: The low activity of muscle fructose-1,6-diphosphatase can be modified by allosteric control. As found with the liver enzyme adenosine monophosphate is a potent inhibitor. In addition high substrate inhibits the activity at physiological pH. This inhibition can be overcome by the addition of allosteric effectors, which are present in the tissues.

Significance: The relief of inhibition of fructose-1,6-diphosphatase activity by compounds which accumulate in fatigued muscle or hypoxic brain permits the gluconeogenic mechanism to operate and thus conserve metabolic reserves.

Proposed Course of Project: These experiments are now in progress. An investigation of the kinetic properties of the fructose-1,6-diphosphatase from red and white muscle and brain will be pursued, especially with relevance to the reversal of glycolytic mechanisms.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/CNC 1884
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Brain glycogen synthetase

Previous Serial Number: NONE

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: David Rottenberg, M.D.

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: To determine the role of glycogen synthetase in glycogen formation in brain in normal and experimental states.

Methods Employed: Two assay methods have been developed to measure glycogen synthetase. Because the activity is so low in brain, very sensitive analytical procedures are required. The first procedure is a modification of a published method. The substrate, uridine diphosphoglucose, is treated to remove contaminating uridine diphosphate. The brain is incubated with uridine diphosphoglucose for the appropriate time and one of the products, uridine diphosphate, is measured by an enzymatic assay system coupled to reduced diphosphopyridine nucleotide. The second procedure is based on the measurement of ^{14}C-labelled glycogen formed when the enzyme is incubated with ^{14}C-uridine diphosphoglucose. The latter method has been adapted from a published technique and modified to increase reproducibility and sensitivity.

Major Findings: The work is in its initial stages. The analytical methods developed will merit separate publication.

Significance: Glycogen synthetase activity in liver, muscle and brain exists in two forms. The enzyme from liver and muscle has been extensively studied and is modified by various hormonal states. The brain enzyme has not been studied in detail. Since glycogen deposition in the brain occurs under various conditions imposed on the experimental animal, it will be of interest to determine the concomitant state of the enzyme.

Proposed Course of Project: Brain enzyme will be purified both in the independent and dependent form and the kinetics studied, especially in relation to the known effectors and the concentration of these effectors in brain. The ratio of the independent to dependent form will be examined in various experimental and endocrine states.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/CNC 1885
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The kinetics of brain glycogen

Previous Serial Number: NONE

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: Hiroshi Watanabe, M.D.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.9
Other:	0.0

Project Description:

Objectives: To determine the rate of glycogen deposition and turnover in brain under normal and experimental conditions. To differentiate between the turnover of the outer tiers and limit dextrin of brain glycogen.

Methods Employed: Brain glycogen will be measured employing enzymatic degradation techniques. Total glycogen will be analyzed using phosphorylase a, amylo-1,6-glucosidase, oligo-1,4 → 1,4 glucan transferase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase. The outer tiers will be measured using 6 times recrystallized phosphorylase a free of debranching complex. The turnover of glycogen will be measured by injecting mice with UL ^{14}C-glucose and counting the labelled glycogen isolated by a KOH-ethanol extraction procedure. The differential turnover of the outer tiers and limit dextrin will be determined by determining the specific activity of one portion of the isolated glycogen sample without further treatment (=total glycogen), and the specific activity of the sample after treatment with crystalline phosphorylast (=limit dextrin).

Major Findings: The work has just been initiated since the arrival of Dr. Watanabe in January 1971.

Significance: Brain glycogen levels increase in various traumatic and experimental states, e.g. anesthesia, radiation, and surgical truma. Subsequent to the anesthesia or injury, the glycogen levels return to normal in most cases. It will be of interest to determine whether newly formed glycogen has a different turnover rate than steady state glycogen. Furthermore no definitive determinations of rates of turnover of cerebral glycogen have been made.

Proposed Course of Project: Once the turnover rate of brain glycogen is determined under normal conditions, the physiological state of the animal will be altered to determine the effect on cerebral glycogen metabolism.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 LNNS/CN 1802
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of Living Nerve Fibers by Differential Interference Microscopy

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.70
Professional:	.60
Other:	.10

Project Description:

Objectives: 1) To examine living peripheral nerve fibers by differential interference microscopy. 2) To observe, in vivo, the effects of fixatives used for electron microscopy. 3) To correlate, in the same myelinated fiber in vivo and electron microscopic observations at intervals during experimental alterations of the axon and myelin sheath.

Methods Employed: Numerous modifications were made in the microscopic chamber used last year. A new perfusion chamber was designed and built in the Plastics Unit of the Instrument Fabrication Unit. Living tadpoles (Xenopus Laevis) were anesthetized and the myelinated fibers in their tales were examined using differential interference contrast (Nomarski).

Sequential observations, in vivo, were continued during fixation for electron microscopy. After embedding in flat epon sheets, the same fibers were identified, sectioned, and examined by light and electron microscopy.

Major Findings: 1) As Speidel showed (1932-1964), single myelinated axons can be identified and traced with their branches for long distances. Those best suited for study were about five microns in diameter. Small, motile, thread shaped structures were observed in these axons on a few occasions. They appeared to move distally and could be axonal mitochondria. Their motility has not yet been documented photographically and their identity remains to be established electron microscopically.

2) *Myelin internodes* appeared smooth except for occasional variations in contour. They were about 150 microns long. Schmidt-Lantermann clefts were not observed.

3) The appearance of axons and their myelin sheaths *remained essentially unchanged during* the early stages of *fixation* for electron microscopy.

4) The *fine structure* of these myelinated fibers is similar to that described in other types of peripheral nerve tissue.

Significance: Although further refinements in chamber design, Nomarski illumination, and photomicrography will be required to achieve the desired resolution, the available results strongly suggest that this *model system* will be useful in elucidating *cellular mechanisms of disease in myelinated nerve fibers*.

An abstract, including these findings, has been submitted for presentation at the Annual Meeting of the American Association of Neuropathologists, June, 1971.

Proposed Course of Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 LNNS/CN 1803
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron Microscopic Study of Axon Schwann Cell Relationships Prior to Myelination in Peripheral Nerves

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: George H. Collins, M.D.

Cooperating Units: None

Man Years:

Total: 2.4
Professional: 1.5
Other: .9

Project Description:

Objectives: 1) To define the geometry of Schwann cells and their surface relationships with axons before the onset of myelination.

2) During this period of development, to stain carbohydrate components of axon and Schwann cell surface membranes and study their distribution by electron microscopy.

Methods Employed: 1) Three dimensional reconstructions were prepared from electron micrographs of transversely sectioned fetal and newborn rat sciatic nerves.

2) Another series of nerves was studied electron microscopically after treatment with alcian blue, ruthenium red, lanthanum hydroxide, and colloidal iron.

Major Findings: 1) In the early stages of development, thin Schwann cell processes surrounded a large bundle of axons which then was invaded and subdivided.

2) The isolation of axons destined to be myelinated occurred first near the ends of Schwann cell processes.

3) Organelles were more numerous at sites of initial mesaxon formation than in other regions of the Schwann cell processes. Subsequent cell division relegated each of the isolated axons to a separate Schwann cell.

4) Surface membrane staining of axons and Schwann cells with alcian blue, etc. has been demonstrated and its distribution is currently being investigated.

Significance: The geometry of Schwann cells and their surface relationships with axons change in an orderly fashion before myelination begins. Abnormalities in this pattern may be important factors in hereditary and developmental neuropathies and the data collected in this study should be useful in interpreting many of the electron microscopic abnormalities that have been reported in human diseases.

Proposed Course of Project: An abstract of the above findings has been submitted for presentation at the June, 1971, Annual Meeting of the American Association of Neuropathologists. The project will be continued.

Honors and Awards: None

Publications:

Observations crucial to the planning of this project and described in NDS(I)-70 LNNS/CN 1804 have been published this year.

Webster, H. deF. and O'Connell, M. F.: Myelin formation in peripheral nerves. A morphological reappraisal and its neuropathological significance. In Proc. VI Int. Congress of Neuropathology. Paris, France, Masson Et Cie, 1970, pp. 579-588.

Webster, H. DeF.: The geometry of peripheral myelin sheaths during their formation and growth in rat sciatic nerves. J. Cell Biol. 48: 348-367, 1971.

Serial No. NDS(I)-68 LNNS/CN 1547
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Ionizing Radiation on the Elasmobranch Brain

Previous Serial Number: Same

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Arland L. Carsten, Ph.D.
Milton Brightman, Ph.D.

Cooperating Units: Medical Department, Brookhaven National Laboratory, Upton, Long Island, N. Y.

Man Years:

Total:	2.3
Professional:	1.7
Other:	0.6

Project Description:

Objectives: Recent studies have shown the shark brain to exhibit a profound and unusual resistance to changes in vascular permeability and blood-brain barrier following severe local injury (Klatzo, I. and Steinwall, O.: Observations on Cerebrospinal Fluid Pathways and Behaviour of the Blood-Brain Barrier in Sharks. Acta Neuropath. 5, 161-175, 1965). The present investigation is to determine if such a resistance would extend also to effects of radiation injury, and if so, to elucidate what factors are responsible for such resistance.

The unique features of cellular morphology such as perivascular arrangement of astrocytes, the ultrastructural characteristics of glia-capillary interphase, lack of tight junctions between the cerebral vascular endothelium cells, distribution of glycogen, etc. create a special interest for studying effects of radiation in Elasmobranchs brain, since it has been shown before that in the mammalian brain the most sensitive changes occur at the glia-blood vessel interphase and involve an abnormal glycogen reaction (Klatzo, et al.: Effects of Alpha Particle Radiation on the Rat Brain Including Vascular Permeability and Glycogen Studies. J. Neuropath. & Exp. Neurol. 20, 459-483, 1961). Also, the very different metabolic and oxygen consumption rates of the shark are of particular interest since the relationship of circulation and oxygen tension levels to severity of radiation injury are well established.

Methods Employed: Approximately 50 nurse sharks have been exposed to a well collimated gamma ray beam directed at the cerebellum and underlying portions of the hypothalamus, tegmentum and pons. The radiation source consisting of the cobalt bomb was shipped from the Brookhaven National Laboratory to the Lerner Marine Laboratory, Bimini, Bahamas, and was installed there. The sharks were subjected to study of acute effects of radiation with dose ranging up to 30,000 r. and were sacrificed within first twelve days following irradiation. Corresponding in dose radiation was given to cats and those were sacrificed at the comparable time intervals. For the observations on the behaviour of the blood-brain barrier, sharks and cats were injected intravenously with the Evans Blue-albumin tracer, usually several hours before the sacrifice. The brains were perfused through the heart by buffered solutions of paraformaldehyde or fixatives designed for the electron microscopy and processed for histological, histochemical and ultrastructural studies. A group of sharks irradiated with 5,000 - 30,000 r. were left in shark enclosures in Bimini for studies on the chronic effects of radiation.

Major Findings: All sharks radiated with 30,000 r. and sacrificed within twelve days after irradiation revealed no evidence of brain injury. There were no apparent histological or histochemical changes, and particularly the picture of histochemical glycogen distribution was no different from control animals. Also, sharks radiated with 30,000 r. and sacrificed 9 months later revealed no abnormalities in the brain. One shark which received 5,000 r. and was sacrificed 2-1/2 years after irradiation showed several small foci of necrosis and astrocytic proliferation in the tegmentum, in the path of the radiation.

Significance to Bio-medical Research and the Program of the Institute: The Section on Comparative Neuropathology, Laboratory of Neuropathology and Neuroanatomical Sciences, is vitally interested in research on elucidation of some basic pathogenic mechanisms in the nervous system. It is felt that studies on primitive vertebrate and invertebrate brain by confrontation of differences found under various experimental conditions offer a unique opportunity to expand our understanding of intricate neurobiological structure-function relationships. This particular project should contribute to elucidation of various patho-mechanisms operative in the radiation damage of the human brain.

Proposed Course of the Project: The project is almost completed. It is intended to extend it by including a brief comparative study on teleost fishes.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/CN 1878
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study on the Basic Aspects of Pathology of the Blood-Brain Barrier (BBB)

Previous Serial Number: None

Principal Investigator: Masaharu Hori, M.D.

Other Investigators: Stanley I. Rapoport, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years:

Total:	2.5
Professional:	1.6
Other:	.9

Project Description:

Objectives: The site of passage restriction for small molecular substances and electrolytes remains unknown although the electron microscopic observations using horseradish peroxidase as a tracer have localized the BBB for proteins as residing in the tight junctions between the cerebral endothelial cells. Also unknown are the routes of abnormal passage of various compounds into the brain parenchyma under pathological conditions. Using strictly standardized models of localized injury to the exposed pial vessels or injury to one hemisphere by unilateral internal carotid perfusion, the following investigations related to some basic aspects of the BBB are undertaken.

1) Hypertonic solutions are applied to produce BBB damage which is reversible and related directly to osmotic effect.

2) Factors affecting the thresholds of osmotic BBB injury, such as the level of systolic blood pressure, hypo- or hyperthermia, etc. are evaluated to elucidate further the mechanisms involved in BBB dysfunction.

3) Applying double tracer method of Oldendorf selective features of BBB vulnerability are ascertained. This data, hopefully, may prove to be useful

for assessment of the size of gaps produced by osmotic shrinkage of endothelium, as well as, for getting insight with regard to routes of passage or transport of various substances including essential nutrients such as glucose and some amino acids.

Methods Employed: Rabbits are used in both models of BBB injury. In the first experiment, the animals are injected with Evans Blue, windows are made exposing cortical surface of one or both hemispheres, circular pledgets of filter paper saturated in various test solutions are placed on the exposed pial surface and kept for 10 minutes. Following this escape of Evans Blue dye from the pial blood vessels is ascertained under the dissecting microscope. In the second type of experiment, the cannula is inserted into one of the common carotid arteries, the external and thyroid branches are ligated so that the injected material is diverted entirely into the internal carotid. Cranial windows are made and intracarotid perfusion is performed under pressure slightly greater than systolic blood pressure (the pial blood vessels blanch). Various BBB damaging solutions are perfused for exactly 10 minutes. The BBB damage is ascertained by extravasation of the Evans Blue in the perfused hemisphere. In application of Oldendorf's method after damaging the BBB through intracarotid perfusion H^3 water and C^{14} test substance are simultaneously injected through the internal carotid. The rabbit is sacrificed by decapitation 15 seconds later and respective radioisotopes are counted in the scintillation counter. The ratio of brain penetration of a test substance is calculated taking H^3 water uptake as 100%.

Major Findings: Application of substances with very limited lipid solubility to the exposed pial vessels revealed a direct reversible osmotic effect on the permeability of the BBB. It was related strictly to osmolality or concentration and not to specific chemical or drug action of substances tested. Reversibility was ascertained by injecting the BBB tracer 30 minutes after cortical pledget application. The surface application of some readily lipid soluble test substances resulted in lesions which appeared to be irreversible indicating that in this situation a structural damage of cell membranes is involved and not merely the separation of the tight junctions.

Significance: Disturbances of the BBB play a paramount role in a number of neurological conditions. Increased vascular permeability allowing escape of serum contents into brain tissue is commonly associated with traumatic brain injuries, brain neoplasms, inflammatory and cerebrovascular lesions. Elucidation of basic BBB phenomena under the normal and pathological conditions, as attempted in this study, may offer a possibility to achieve some control of vascular permeability and to diminish adverse effects of "leaky" blood vessels on the brain parenchyma.

Proposed Course of Project: The experiments on pial blood vessels are well advanced and an appreciable amount of data has been already collected. The experiments on carotid perfusion injury and those with application of double tracer method of Oldendorf are still in preliminary stages.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 LNNS/ENP 944
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hematologic control of primates

Previous Serial Number: SAME

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.0
Other:	0.3

Project Description:

Objectives: Hematologic control of caged primates.

Methods Employed: Different primate species have been regularly examined by various hematologic techniques; weight and dietary regimen were controlled.

Major Findings: Many of the primates were found to suffer from severe infections and aberrations of blood composition.

Significance: A careful hematologic examination has been found to be significant in order to estimate the health condition prior to subjecting primates to experiments.

Proposed Course of Project: To prepare the data for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 LNNS/ENP 1065
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title:	Perfection of the perfusion technique for fixation in situ.
Previous Serial Number:	SAME
Principal Investigator:	Jan Cammermeyer
Other Investigators:	None
Cooperating Units:	None
Man Years	0
Project Description:	This project has been terminated.

Serial No. NDS(I)-63 LNNS/ENP 1066
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Acute degenerative changes in the central nervous system.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.6
Professional: 0.1
Other: 0.5

Project Description:

Objectives: To ascertain the specificity of neuronal changes.

Methods Employed: Cerebral necrosis is obtained by embolization of blood vessels or constriction of arteries and by compressing cerebral surface. The tissues are preserved by the submerged heart perfusion procedure.

Major Findings: Preliminary study has disclosed that by the use of current procedures of preservation, the tissue can not yet be regarded as adequately fixed. This is evident by the fact that some of the changes are not distinguishable from non-specific changes.

Significance: So as to be able to interpret properly the changes encountered in neuropathologic material, it is of paramount significance that the appearance of non-specific changes be fully recognized.

Proposed Course of Project: Preparation of additional material by current methods for light microscope. As part of a long-term study on the occurrence of non-specific cell changes of artifactual nature, the observations will be included in a comprehensive review.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/ENP 1449
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of cortisone on the retrograde neuronal changes

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.8
Professional: 0.2
Other: 0.6

Project Description:

Objectives: To determine what effect cortisone treatment may have on the acute reactive phase and the subsequent recovery phase of motor neurons subsequent to transection of their axons.

Methods Employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-gallocyanin method. New material is being prepared for use of other staining methods.

Major Findings: Preliminary studies have revealed that the acute peripheral chromatolysis is not influenced by cortisone treatment although the incidence of reactive mitosis is depressed. However, because of subtle differences in the neuronal reaction, new material has been prepared for supplementary studies.

Significance: In the acute stage of retrograde changes, cortisone has been found to have a selective action on the mesodermal elements. The results of this study would form a basis to evaluate the usefulness of cortisone treatment during conditions of nerve regeneration.

Proposed Course of Project: Histologic preparation of newly prepared material to study in particular whether in cortisone treated animals the sequence of retrograde reaction is different from that in untreated material, with respect to content of RNA and glycogen in neurons as well as reactability of microglial cells.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/ENP 1676
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A comparative study of the area postrema and the caudal part of the fourth ventricle.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine species differences in the topographic and histologic characteristics of the area postrema and surrounding fourth ventricle and to determine the effect of chronic meningitis on the topography of the roof of the fourth ventricle.

Methods Employed: Light microscopic techniques.

Major Findings: 1) The size and topography of the area postrema, as well as its permeability to trypan blue, have been found to vary greatly with species. Even within the same species, the caudal pole of the area postrema may terminate either dorsal to a commissure of the alae cinereae or in the wall of a more ventrally situated central canal, as in the cynomolgus monkey or the cat. In birds, such as the pigeon, the area postrema is not developed, while in mammals, it is large and Y-shaped or small and massed into a median structure. In most rodents, varying in size from the 25 g mouse to the 9 kg paca, the area postrema consists of a large median mass, but in other rodents, such as the squirrel, the same region is bilateral. In the capuchin monkey, the area postrema does not extend as far caudally to the commissure of the alae cinereae as in other monkeys.

2) The extent of the area postrema surface which is covered by leptomeninges or exposed to the lumen of the fourth ventricle or of the central canal varies greatly from species to species and also among individuals of the same species, such as the cynomolgus monkey and the cat.

3) The fourth ventricle terminates at the level of the area postrema in some species, but in others extends caudally in a dorsal cavity over the area postrema and even beyond this region. In a few species, such as the guinea pig and the squirrel monkey, the caudal part of the fourth ventricle communicates with the cerebello-pontine cistern through irregular apertures in the roof. In other mammals, no such aperture is demonstrable.

Significance: For correct interpretation of the functional significance of the area postrema as well as the physiology of the cerebrospinal fluid, accurate information about topographic peculiarities is of paramount significance. At this stage of experience, it can merely be stated that because of the considerable species differences, one must be cautious in generalizations about the functional role of the area postrema and the circulation of the cerebrospinal fluid. However, knowledge of these differences is necessary for proper planning of a surgical experiment and the selection of appropriate animal.

Proposed Course of Project: To examine the area postrema under different experimental conditions. To acquire animals suffering from hydrocephalus and examine the brain stem in serial sections.

Honors and Awards: None

Publcations:

Cammermeyer, J.: Median and caudal apertures in the roof of the fourth ventricle. J. Comp. Neur. 141, 499-519, 1971.

Serial No. NDS(I)-69 LNNS/ENP 1677
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparative anatomical study of mast cells in the area postrema.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.1
Other:	0.8

Project Description:

Objectives: To determine the significance of mast cells in the area postrema.

Methods Employed: Histologic methods for identification of mast cells.

Major Findings: A preliminary review of the area postrema in more than 10 species has revealed considerable individual and species differences in content of mast cells. These cells are extremely numerous in some species, such as the cynomolgus and the speciosa monkeys, whereas others, such as the mulatta and squirrel monkeys, have very few or none. The dog and the cat have many mast cells, the dog more than the cat but far less than the monkeys.

Significance: Because of the larger number of mast cells in the area postrema, albeit in a few species only, the mast cells must be considered to play an important role in the physiology of the area postrema. On the basis of these studies, only a few species can be regarded as useful for further experimental work.

Proposed Course of Project: To establish the reaction of mast cells to drugs administered to the animal by various routes and to ascertain whether changes in histologic characteristics of the area postrema take place.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 LNNS/ENP 1807
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Myelencephalic bodies in the roof of the fourth ventricle and the surface of the medulla oblongata in the guinea pig.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years 0

Project Description: This project has been terminated.

Serial No. NDS(I)-71 LNNS/ENP 1882
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Assessment of artifactitious changes in central nervous system.

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 2.2
Professional: 0.5
Other: 1.7

Project Description:

Objectives: To determine the origin of certain artifactitious changes in the central nervous system and to search for methods to eliminate them.

Methods Employed: Animals used in various experiments - axotomy, irradiation, injection of different drugs, interruption of cerebral circulation, swimming - and control animals of different ages have been prepared by different methods of fixation.

Major Findings: 1) When tissues have not been adequately fixed, neurons and glial nuclei undergo non-specific changes which are induced by post-mortem trauma.

2) Regardless of methods of fixation, metachromatic plaques with non-specific "demyelination" occur ubiquitously, but with a tendency to accumulate near sites of post-mortem trauma.

3) A number of abnormalities in stainability of tissues are of uncertain origin.

4) In radiation material, reaction of cerebellar granule cells, referred to as "nuclear pyknosis," is different from degenerative changes of mitotic cells observed in regions with intense cellular proliferation. For the latter reaction, I have coined the term "dysmitosis" to distinguish it from ordinary cell necrosis.

Significance: For a correct appreciation of maladies in clinical neuropathology and of changes induced by experiments, as e.g. for study of drug effects, it is necessary to have a clear concept of the specificity of tissue changes and to this end the characteristics of non-specific or artifactitious changes must be recognized. A review of personal material prepared under known conditions has demonstrated that in a number of publications artifactitious changes have been confused with specific changes.

Proposed Course of Project: Review of material assembled over a period of more than 15 years, execution of supplementary experiments, selection of characteristic observations for photomicrography, listing of pertinent literature, and editing of the manuscript.

Honors and Awards: None

Publications:

Cammermeyer, J.: A light microscopic study of microglial cells: Mitosis, development and proliferation. In VI. Congrés international de Neuropathologie, Paris, 31 aout-4 septembre 1970. Paris, Masson & Cie., 1970, pp. 424-436.

Serial No. NDS(I)-60 LNNS/FN 712
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The ascending and descending auditory connections in the primates

Previous Serial Number: Same

Principal Investigator: Grant L. Rasmussen

Other Investigators: Prof. Jean Desmedt, Universite Libre de Bruxelles, Brussels, Belgium

Cooperating Units: Universite Libre de Bruxelles, Brussels, Belgium

Man Years 0

Project Description: This project has been terminated.

Serial No. NDS(I)-65 LNNS/FN 1229
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Fine structure of afferent and efferent nerve endings in the cochlear nucleus of normal and experimental animals

During FY 1971 this project was incorporated with Serial No. NDS(I)-65 LNNS/FN 1231

Serial No. NDS(I)-65 LNNS/FN 1231
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Organization of synaptic fields in the mammalian brain

Previous Serial Number: Same and incorporating Serial No. NDS(I)-65 LNNS/FN 1229

Principal Investigator: Thomas S. Reese

Other Investigators: Edward White

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To determine the types of anatomical and functional relationships between synapses in the vertebrate brain. We have concentrated our efforts, to date, on the central auditory and olfactory pathways.

Methods Employed: Appropriately prepared brains are examined by transmission microscopy. Three dimensional reconstructions based on electronmicroscopical examination of serial sections are correlated with the results from examining Golgi impregnated brains with the light and electron-microscopes.

Major Findings: Our work has showed reciprocal synapses between dendrites of mitral (multipolar) and granule (axonless) neurons in the olfactory bulb. These reciprocal synapses are excitatory in a mitral-to-granule direction and inhibitory in a granule-to-mitral direction. Recent work in another layer of the olfactory bulb shows a hierarchial organization of nonreciprocal synapses.

Significance: Much effort in neurological research has been directed to mapping the axonal tracts connecting different areas of cerebral grey matter. Our effort is directed toward understanding what occurs at the termination of these tracts, where we have shown that dendritic synaptic interactions may result in such phenomena as lateral and recurrent inhibition or excitation. Such knowledge about the types of synaptic interactions in different regions of the central neurons will form a basis for understanding the effects of various neuropharmacological agents on the mammalian brain.

Proposed Course of Project: A paper covering some of our recent work in this area will be published in "The Synapse", G. D. Pappas, ed. Serial sectioning will continue to be used extensively to determine the sources and types of dendritic synapses in the olfactory bulb. It is expected, in the future, to apply a similar approach to other areas, such as the auditory system.

Honors and Awards: None

Publications:

Reese, T. S., and Brightman, M. W.: Olfactory surface and central olfactory connections in some vertebrates. In Loewenstein, O. (Ed.): Mechanisms of Taste and Smell in Vertebrates. London, J. & A. Churchill Ltd., 1970, pp. 115-149.

Serial No. NDS(I)-67 LNNS/FN 1442
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Permeability of cellular layers in the vertebrate nervous system

Previous Serial Number: Same and incorporating Serial No. NDS(I)-69 LNNS/FN 1683

Principal Investigator: Thomas S. Reese

Other Investigators: Milton W. Brightman, Sachio Nabeshima, Randolph Perkins and William Friedel

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.4
Other:	0.6

Project Description:

Objectives: Determination of the pathways taken by colloids and large molecular weight solutes to cross the various cellular layers associated with the nervous system. Currently being studied are: 1. ependyma covering the median eminence and area postrema 2. arachnoid-dural membrane 3. arachnoid villae 4. epithelium and vessels of the stria vascularis in the middle ear 5. epineurium, perineum, and vessel walls in peripheral (sciatic) nerve.

Methods Employed: The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase (MW 40,000) after it is injected into various brain or blood compartments of living, anesthetized animals. The intercellular junctions fronting these compartments are studied by the colloidal lanthanum or uranyl acetate methods. A new peroxidase of low molecular weight (microperoxidase MW 1800) is now being used.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach the generally open interstitial spaces of the brain. However, peroxidase does not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of "tight" junctions within the specialized epithelial cells lining the ventricular surface over these regions. The use of a smaller peroxidase has allowed it to be visualized crossing the capillary pores in the median eminence and area postrema.

A specialized layer of cells joined by tight junctions at the outer border of the arachnoid also prevents peroxidase from reaching the subdural space. This phenomenon and the outlets for cerebrospinal fluid at nerve roots and arachnoid villae are being studied by Dr. Nabeshima.

In peripheral nerve, the endothelial cells of endoneurial capillaries are joined by tight junctions which exclude peroxidase from the surrounding endoneurium. Likewise, the inner layers of the perineurial sheath are joined by tight junctions which exclude from the endoneurium peroxidase that has been injected into the nerve or has leaked out of the perineurial blood vessels subsequent to intravenous injections of tracer.

In the stria vascularis, the vascular endothelium is permeable to peroxidase but the epithelium fronting the endolymph is impermeable at the times and concentrations we used.

Significance: We are determining the permeability to a small protein of the various cellular layers associated with the mammalian nervous system. Our determinations are by means of cytological techniques that show specifically which components of which layers are permeable. Thus we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain (blood-brain barrier).

Proposed Course of Project: The work on the arachnoid membrane will be presented to the annual meeting of the American Association of Anatomists and the American Association of Neuropathologists. The work on the arachnoid villae and stria vascularis is continuing and that on the area postrema and median eminence awaits publication. The work on the peripheral nerve is completed and published but we are planning to repeat some of the experiments using microperoxidase.

Honors and Awards: None

Publications:

Olsson, Y., and Reese, T. S.: Permeability of vasa nervorum and perineurium in mouse sciatic nerve studied by fluorescence and electron microscopy. J. Neuropath. Exp. Neurol. 30: 105-119, 1971.

Serial No. NDS(I)-69 LNNS/FN 1683
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ultrastructural basis of the blood-nerve barrier

During FY 1971 this project was incorporated with Serial No. NDS(I)-67 LNNS/FN 1442

Serial No. NDS(I)-69 LNNS/FN 1684
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structure and function of close intercellular (gap) junctions

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: To study the structure and distribution of gap junctions and to show directly with the electronmicroscope their permeability to inter and intracellular tracers.

Methods Employed: Peroxidases of various molecular weights are applied to or injected into cells joined by gap or tight junctions and the peroxidase is subsequently localized with the electronmicroscope. The peroxidases were developed by Dr. Ned Feder, NIAMD, who is supplying them for this study. Over the past year new methods have been discovered for more sensitive localization of peroxidase activity.

Major Findings: Microperoxidase (MW 1800) (but not horseradish peroxidase (MW 40,000) injected into a crayfish giant axon crosses to a neighboring giant axon,) to which it is joined by gap junctions. This movement of microperoxidase occurs only under conditions where the axons are electrically coupled. When a coupled preparation is not injected but soaked in microperoxidase, no tracer is detected within either of the axons. At the gap junctions between coupled injected axons, bridges 10-15 Å in diameter cross the gap. These bridges may localize the channels through which microperoxidase crosses from axon to axon.

Significance: Intercellular coupling has recently been shown to be important in a variety of normal cellular junctions. Changes in coupling appear to be a component of cancerous changes. Our study is an attempt to provide a morphological basis for intercellular coupling by: 1. defining whether this communication occurs directly or via the intercellular spaces; 2. defining what size materials can pass from one cell to adjacent cells; 3. perhaps demonstrating directly the cell-to-cell channels that have been thought to be present within gap junctions. We elected to work on the crayfish because the large size of its nerve cells makes it possible to do this experiment with presently available techniques.

Proposed Course of Project: Preliminary results will be presented at the annual meeting of the American Association of Anatomists in April. Further experiments are needed to complete our results.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 LNNS/FN 1806
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Transynaptic degeneration in the medial superior olivary nucleus

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese

Other Investigators: Randolph Perkins

Cooperating Units: None

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To determine transynaptic degenerative changes in synaptic endings after removing the excitatory synaptic input to the cell bodies giving rise to these synaptic endings. These changes will be studied at different ages.

Methods Employed: The cochlea is removed from chinchillas at various ages and the synaptic endings of axons originating in the cochlear nucleus and ending on dendrites in the medial superior olive are studied after various periods of time.

Major Findings: None

Significance: These experiments may show the effect of synaptic input to a cell on the structure and organization of the synapses it forms on other cells. These experiments can be done at early ages when, in the visual system at least, it has been shown that synaptic connections are being organized at certain critical ages. Such results might have a bearing on the problem of early treatment of congenital deafness.

Proposed Course of Project: Dr. Perkins was unable to join this project on a full time basis. This project received no time this year. It will be kept open next year with the hope that help will be available to complete it.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/FN 1881
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structural basis of synaptic transmission

Previous Serial Number: None

Principal Investigator: John Heuser

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To determine the structural changes which accompany physiological changes at nerve-muscle synapses.

Methods Employed: Frog sartorius nerve-muscle preparations are exposed to altered ionic environments or stimulated with various programs, and their responses are monitored by standard electrophysiological means until they reach a desired functional state. Then they are immediately fixed and prepared for examination with the electron microscope. Random views of neuromuscular junctions are analyzed by stereoscopic means in search of statistically significant structural changes.

Major Findings: The traditional fine-structural appearance of the synapse represents a resting or equilibrium state. With intense electrical stimulation and transmitter release approaching exhaustion the intracellular organelles of the presynaptic terminal undergo well-defined, reversable changes. These include mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus-like bodies, apparently as intermediate stages in the recycling of membrane back into synaptic vesicles.

Significance: First, this work contributes to an understanding of the role of membranes in the storage and release of transmitter at synapses.

Second, it helps to broaden the general anatomical criteria of synapses, thereby aiding future attempts to define the existence and location of functional contacts in the CNS, and most important, permitting attempts to define their physiological state.

Proposed Course of Project: The present preparation and approach will be adhered to strictly, in order to insure the reproducibility and statistical significance of the results to date, and to allow comparison with future correlations of structure and function of neuromuscular synapses under altered metabolic or environmental states, including metabolic poisons, inhibitors of transmitter and membrane synthesis, and naturally occurring neuromuscular blocking proteins such as Botulinus toxin and Bungarotoxin.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/NC 1879
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemical Changes in Brain of Experimentally Induced Micrcencephaly

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Gert L. Laqueur, M.D.
Igor Klatzo, M.D.
Stanley Rapoport, M.D.
Masaharu Hori, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.7
Professional:	1.0
Other:	.7

Project Description:

Objectives: Recently transplacental chemical microencephaly was induced with methylazoxymethanol MAM, the aglycone of the naturally occurring glucoside cycasin, and the synthetic methylazoxymethyl acetate MAMA, in rats. The microencephaly was associated with decreased intellectual capacity and development of gliomas. (Spatz, M. and Laqueur, G. L.: Chemical induction of microencephaly in two strains of rats. Proc. Soc. Exp. Biol. Med. 129: Part I, 705-710, 1968.)

The present investigation has been a continuation of comparative study to analyze the chemical composition of brain from microencephalic rats and of those from normal rats during development.

Methods Employed: The microencephaly was induced by a single intraperitoneal or intravenous injection of 20 mg/kg of MAM or MAMA on the 14th or 15th day of gestation. The control animals were treated similarly but received saline instead of MAM. The offsprings (of both the experimental and control rats) were sacrificed on the designated postnatal dates, and the entire brain of each animal was first removed, then divided into two parts, the cerebral hemispheres, and the remaining brain. The samples from each part were weighed and pooled according to age and sex of the animals, for the determination of total lipids, phospholipids, and cholesterol. The lipid extraction was carried out according to the method of Folch, et al., (J. Biol. Chem. 226: 497-509, 1957). The Fiske & Subborow method was used for the phosphorus lipid analysis, (J. Biol. Chem. 66: 375-400, 1925), and the total cholesterol was determined by the method of Sperry, et al., (J. Biol. Chem. 187: 97-110, 1950).

Major Findings: The striking feature of chemically induced microencephaly was a uniform reduction in brain weight due to diminution of both cerebral hemispheres in all animals within each litter. The arrest of brain growth occurred at 14 days postnatally when the cerebral lipids normally increase. Thus the amount of the total lipids in the cerebral hemispheres was found to be diminished by 25% when compared with that of control on a wet and dry basis. The total phospholipids were found significantly lower in the cerebral hemispheres of 7-270 days old, and in the remainder of the brain only from 60-270 days old microencephalic rats, when compared with those of normal animals, both on a wet and dry basis. The cholesterol values didn't differ in the two groups.

Significance to Bio-medical Research and the Program of the Institute: The Section on Neurocytobiology, Laboratory of Neuropathology and Neuroanatomical Sciences, is interested in the study of placental and blood-brain barrier (BBB) in order (1) to elucidate some basic mechanism responsible for selective passage of physiologic and toxic substances accountable for normal and abnormal development of the central nervous system, and (2) to investigate the effects of toxic substances on developing brain. It is felt that these studies will contribute to a better understanding of basic placenta and BBB phenomena, and its relationship to the normal and pathologic function of the brain. These investigations will serve also as model systems for the study of human brain function in health and disease.

Proposed Course of Project: Future investigations of microencephalic rats should be concerned with (a) fractionation of phospholipids and analysis of myelin in the brain, (b) the investigation of carbohydrate metabolism during the development of the brain, and (c) testing of MAM effect on chromosomes in fetal brain *in vitro*.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/NC 1880
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Brain Uptake of Radioactive Substances during Reversible Osmotic Injury of Blood-Brain Barrier (BBB)

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Gert L. Laqueur, M.D.
Igor Klatzo, M.D.
Stanley Rapoport, M.D.
Masaharu Hori, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.7
Professional:	1.0
Other:	.7

Project Description:

Objectives: Currently a model system for reversible blood-brain barrier (BBB), injury was developed in this laboratory (Rapoport, S., Hori, M., and Klatzo, I. - personal communication).

The present investigation is designed to apply a double isotope tracer technic of Oldendorf to assess any selective permeability changes with regard to various physiologically important compounds, which may occur under the condition of our model for BBB injury.

Methods Employed: The application of the Oldendorf method described for rats (Oldendorf, W. H., Brain Research 24: 372-376, 1970) is being tested and modified for rabbits in order to evaluate the brain uptake of double labeled substances injected into the internal carotid artery. The injected mixture consists of [H^3] water (as an internal reference standard of brain uptake), and a test C^{14} labeled compound, which permits to measure the ratio of the test substance to water uptake into the entire brain or into the selective regions of the brain in the experimental animals.

Major Findings: None yet.

Significance to Bio-medical Research and the Program of the Institute: The Section on Neurocytobiology, Laboratory of Neuropathology and Neuroanatomical Sciences, is interested in (1) the study of the blood-brain barrier (BBB) in order to elucidate some basic mechanism responsible for selective passage of physiologic and toxic substances into the mature brain under normal and pathological conditions; (2) to investigate the effects of chemotherapeutic as well as toxic compounds on the brain. It is felt that these studies will contribute to a better understanding of basic BBB phenomena, and its relationship to the normal and abnormal function of the brain. These investigations will serve also as model systems for the study of human brain function in health and disease.

Proposed Course of Project: To modify and apply Oldendorf technic using double labeled tracers to assess the type and degree of reversible osmotic injury of blood-brain barrier, BBB.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-66 LNNS/NC 1304
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pinocytosis in the brains of dead rats

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

This project is being held in abeyance as the work will have to be reinvestigated with new staining techniques and a new marker (Peroxidase).

Serial No. NDS(I)-67 LNNS/NC 1443
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Others:	0.2

Project Description:

Objectives: To examine morphologically the blood-CSF barriers of the choroid plexus to proteins.

Methods Employed: Horseradish peroxidase (HRP) is injected intravenously at the same time that ferritin is perfused through the cerebral ventricles in mice.

Major Findings: The basal and lateral parts of the cell membrane face the blood space and pinocytose appreciable amounts of HRP whereas the apical parts pinocytose only a small fraction of the ferritin. The same vacuole receives both HRP and ferritin.

Significance: Different parts of the same cell membrane belonging to choroidal epithelium have different ability to pinocytose proteins.

Proposed Course of Project: To extend the observations on movement of peroxidase both from the blood and from the CSF and to determine the role of vesicular transport across the fenestrated endothelium.

Honors and Awards: None

Publications:

Brightman, M. W.: Choroid plexus structure as related to the blood-cerebrospinal fluid barrier to protein. In M. Netsky (Ed.): *The Choroid Plexus and Ependyma in Health and Disease*. (In press)

Serial No. NDS(I)-68 LNNS/NC 1587
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A blood-brain-barrier to peroxidase in the normal and injured brain of elasmobranchs

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: Thomas Reese and Igor Klatzo

Cooperating Units: None

Man Years

Total:	1.2
Professional:	0.6
Others:	0.6

Project Description:

Objectives: To determine the final structural basis for the blood-brain-barrier to peroxidase in the shark.

Methods Employed: Exogenous (horseradish) peroxidase is injected into the caudal vein or cerebral ventricles of nurse sharks and the ultimate distribution of the protein is detected electronmicroscopically by a cytochemical method.

Major Findings: The junctions between adjacent endothelial cells of cerebral capillaries are open in the shark instead of closed, as in mammals and amphibians. Peroxidase is then able to pass between endothelial cells to enter the capillary basement membrane as far as tight junctions between perivascular glial end feet.

Significance: The elasmobranch is to date exceptional in that the blood-brain-barrier to protein is glial rather than endothelial. The site of these tight junctions thus represents a fundamental difference between mammals and the elasmobranch.

Proposed Course of Project: The findings are being confirmed and have been extended to another elasmobranch (guitar fish) and a teleost (goldfish).

Honors and Awards: None

Publications:

Brightman, M. W., Reese, T. S. and Feder, N.: Assessment with the electronmicroscope of the permeability to peroxidase of cerebral endothelium and epithelium in mice and sharks. In Crone, C. and Lassen, N. A. (eds.): Capillary Permeability. Copenhagen, Munksgaard, 1970, pp. 468-476.

Brightman, M. W., Reese, T. S., Olsson, Y. and Klatzo, I.: Morphological aspects of the blood-brain-barrier to peroxidase in elasmobranchs. Progress in Neuropathology (In press)

Serial No. NDS(I)-69 LNNS/NC 1678
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Distribution of exogenous proteins in brain tumors

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: L. Prescott, T. S. Reese, N. Vick, and D. Bigner

Cooperating Units: Medical Neurology Branch, NINDS

Man Years

Total:	0.5
Professional:	0.3
Others:	0.2

Project Description

Objectives: To determine differences in permeability of the endothelium in neoplasms to peroxidase and the availability of the extracellular spaces (ecs) of the tumors to this protein.

Methods Employed: Rous sarcoma virus (RSV) or Simian virus 40 (SV_{40}) are inoculated into the brains of newborn hamsters and, after the appearance of ataxia, circling or somnolence, peroxidase is injected intravascularly and the fixed tumor examined electronmicroscopically.

Major Findings: Peroxidase rapidly crosses the RSV tumor capillaries and is avidly pinocytosed by "dark" tumor cells whereas light, (glial?) cells pinocytose very little. Most of the capillaries have tight junctions. In the SV_{40} tumor, most of the capillaries are fenestrated and the peroxidase crossing them is pinocytosed by more than one type of tumor cell.

Significance: The fenestrae of tumor capillaries appear to be one route of entry of protein from blood to tumor extracellular space.

Proposed Course of Project: To compare the entry routes and distribution of protein in the two types of brain tumors.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1805
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A reinvestigation of central nervous system fine structure with freeze-replication techniques

Previous Serial Number: Same

Principal Investigator: Marie Shatos

Other Investigators: M. W. Brightman and T. S. Reese

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To reinvestigate the fine structure of the central nervous system with freeze-replication techniques.

Methods Employed: In a departure from commercially available freeze-fracture evaporators which include oil-diffusion pumps of limited capacity, a system of ion-diffusion pumps and freezing stage is being assembled. This system, since it is free of oil, will greatly diminish hydrocarbon contamination and is capable of a vacuum higher than the presently available systems.

Major Findings: Preliminary results indicate that even with conventional pumps, a replica showing the three-dimensional arrangement of afferent olfactory nerve processes is obtained.

Significance: By avoiding the process of plastic embedding, and ultimately, of chemical fixation, a three-dimensional replica of cells and their interconnections that is closer to the living state, becomes feasible.

Proposed Course: To re-examine intercellular junctions after aldehyde fixation only and, eventually, without such fixation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 LNNS/TNF 1054
1. Neuropathology and Neuroanatomical Sciences
2. Section on Trophic Nerve Function
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Trophic functions of the peripheral nervous system

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers

Cooperating Units: Laboratory of Neurochemistry, NINDS

Man Years:

Total:	2.4
Professional:	1.0
Other:	1.4

Project Description:

Objectives: To investigate the mechanisms by which the nerve regulates the metabolism of the muscle fiber.

Methods Employed: Frozen sections of mammalian muscle have been incubated by histochemical techniques to reveal the ATPase, succinic dehydrogenase and cholinesterase activities of the various muscle fibers. The low molecular weight subunits have been isolated from myosin and examined electrophoretically.

Major Findings: Although denervation produces changes in the enzyme activities of various fibers, and causes a slowing of contraction speed, it does not cause any alteration in the characteristic subunits of the slow and fast muscle myosin. Muscle fibers have generally been classified into three major fiber types according to their histochemical reactions. We now observe that when serial sections of the same fiber are incubated for a variety of different enzymes, considerably more than three classes of fibers occur. When the muscle is forced to perform various kinds of work the fibers undergo a variety of profound alterations in their histochemical profile.

Significance: The muscle fiber is a dynamic cell, capable of undergoing remarkable alterations in its phenotypic appearance in response to a variety of environmental influences. Major among these is the effect of denervation, cross-reinnervation and exercise. Because of these changes it is apparent that to classify muscle cells into "fiber types" on the basis of such readily alterable phenotypic characteristics will prove misleading for histological and pathological investigations.

Proposed Course of Project: To be continued and extended during the coming year.

Honors and Awards: None

Publications:

Samaha, F. J., Guth, L., and Albers, R. W.: The neural regulation of gene expression in the muscle cell. Exptl. Neurol. 27: 276-282, 1970.

Guth, L. and Samaha, F. J.: Procedure for the histochemical demonstration of actomyosin ATPase. Exptl. Neurol. 28: 365-367, 1970.

Guth, L. and Dempsey, P. J.: Mechanisms controlling glycogen levels in injured brain, normal brain, liver, and heart. Exptl. Neurol. 29: 152-161, 1970.

Serial No. NDS(I)-66 LNNS/TNF 1303
1. Neuropathology and Neuroanatomical Sciences
2. Section on Trophic Nerve Function
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Histo- and biochemical characteristics of muscle sensori - motor components

Previous Serial Number: Same

Principal Investigator: Herbert Yellin

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.4
Professional:	1.0
Others:	0.4

Project Description

Objectives:

1. To study by histochemical methods the comparative aspects of muscle metabolism in several mammalian species.

2. To demonstrate by histochemical methods the plastic nature of muscle fiber metabolism in response to exercise and accompanying conditions.

3. Attempt to discern the differences in the neuroregulatory and myoexpressive factors of skeletal and fusimotor units.

Methods Employed:

1. Normal muscles of a variety of mammalian species, e.g., rat, mouse, guinea pig, rabbit, cat and man, were studied histochemically for selected enzymes of oxidative and glycolytic metabolism and for their energy-expending actomyosin ATPase activity.

2. Enzyme histochemical techniques were applied to rat muscles that had, by virtue of excision of their synergists,

been required to perform excessive and unusual work.

3. Changes in the enzyme histochemical properties of extra- and intrafusal musculatures were sought following cross- and random reinnervation of specific hindleg muscles. Initial efforts were made to reinnervate at the level of the spinal roots, and thereby obviate the need for disturbing both afferents and efferents in the same preparation.

Major Findings:

1. In support of earlier findings, the results suggested additional interspecies differences in the enzyme histochemical properties of muscle fibers from comparable muscles.

2. Additional evidence that the hitherto widely utilized two or even three "fiber type" classifications of muscle fibers are restrictive and not truly representative of the wide range of properties that muscle fibers adopt while adjusting to changing conditions.

3. Skeletal muscle fibers are capable of adapting rapidly and differentially to altered functional demands as revealed by the profound alterations in enzyme histochemical and structural properties of the affected cells.

4. The enzyme histochemical properties of both extra- and intrafusal (mammalian) skeletal muscle fibers can be altered by alien reinnervation during the early neonatal states of development in the rat. However, although the extrafusal musculature retains this metabolic plasticity throughout life, the intrafusal musculature appears to become refractory to change with the onset of maturity.

Significance:

1. The subtle discrepancies in histochemical properties of skeletal muscle fibers of different mammalian species may derive from the distinctive gaits and nature of confinement of the several common laboratory animals (including man).

These histochemical discrepancies between species presumably have biochemical counterparts, and the latter may be relevant in comparative studies of normal, exercised and diseased muscle.

2. Mammalian skeletal muscle fibers are metabolically malleable, and exhibit differential responses to exercise and the shortening and/or stretching that accompanies such imposed regimens. Further studies on the factors regulating the basic

metabolic properties of muscle are necessary before the significance of findings in diseased muscles can be fully appreciated.

3. The differential responses of adult extra- and intrafusal muscle to altered function, altered innervation or disease, with sparing of the intrafusal musculature in most instances, suggests that some regulatory factor(s), i.e. afferent and/or efferent innervation, hormones, etc., may impart a stable and infrequently disordered metabolism to the intrafusal musculature.

Proposed Course of Project:

These studies will continue as a major portion of the coming year's work.

Honors and Awards: None

Publications:

Yellin, H. and Eldred, E.: Spindle activity of the tenotomized gastrocnemius muscle in the cat. Exptl. Neurol. 29: 513-533, 1970.

Serial No. NDS(I)-68 LNNS/TNF 1586
1. Neuropathology and Neuroanatomical Sciences
2. Section on Trophic Nerve Function

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Trophic nerve function

Previous Serial Number: Same

Principal Investigator: Andrew A. Zalewski

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1.6
Professional: 1.0
Others: 0.6

Project Description:

Objectives: Previous studies have demonstrated that peripheral or central fibers of gustatory neurons can cause taste bud regeneration and that chromatolysis of the gustatory neurons did not interfere with their function. These findings coupled with the observation that some neurons in sensory ganglia can survive transplantation prompted a series of experiments to determine whether transplanted gustatory neurons would survive, regenerate their nerve fibers, and retain the trophic property of inducing taste bud formation. Autologous and homologous transplantation was performed so that the role of the immune system regarding neuron survival and function could be evaluated.

Another aspect of neuron function that was investigated was to determine whether the trophic function of nerve to muscle was specific as was shown for the gustatory neurons regarding taste bud regeneration. The question asked was: can any nerve (e.g., sensory or autonomic) functionally innervate skeletal muscle or is this innervation a specific property of the motoneuron.

Methods Employed:

1. Autologous and homologous transplantation of the rat

vagal nodose ganglion (fibers innervate taste buds in the pharynx) was performed and the peripheral fibers of the ganglion joined to the cut distal glossopharyngeal nerve to see if vagal neurons would survive, regenerate their nerve fibers down the glossopharyngeal nerve pathway, and cause the formation of taste bud in the tongue's vallate papilla. Light microscopical and histochemical studies were performed to detect neuron survival, nerve regeneration, taste bud regeneration, and any immune cellular response.

2. The rat skeletal sternomastoid muscle was denervated and chronically denervated muscle compared to denervated muscle into which motor, sensory or autonomic (preganglionic cervical sympathetic) nerves were implanted. Tests were performed on the muscle months later to determine if any functional reinnervation (e.g., restoration of muscle weight, contraction response to nerve stimulation, etc.) occurred.

Major Findings:

1. Some neurons in the autologous and homologous transplanted nodose ganglion survived, grew out their nerve fibers, and caused taste bud regeneration. This occurred even when a cellular immune response was present around the homologously transplanted tissue.

2. Functional reinnervation of denervated muscle occurred only with motoneuron and not with sensory or autonomic reinnervation.

Significance:

1. The above findings mean that nervous tissue can be successfully transplanted and remain functional. The observation that neurons can survive and function after homologous transplantation suggests that neurons do not evoke or are not adversly affected by an immune response.

2. The finding that only motoneurons can functionally innervate muscle demonstrates the specificity of mammalian nerve function. Furthermore, since the cholinergic sympathetic nerves did not innervate denervated muscle the role of acetylcholine as the "trophic agent" merits further investigation.

Proposed Course of Project:

1. Transplantation studies will be performed to further

evaluate the antigenicity and response of neurons to the immune reaction. Other nerve tissue, such as spinal motoneurons, will be transplanted to determine if other nerve tissue can survive and function.

2. Studies concerning central nervous system regeneration will be undertaken in hopes that intergrated function of the transplanted tissue with higher host brain centers can be formed.

3. Future studies determining the mechanism of the specificity of end-organ innervation will also be performed.

Honors and Awards: None

Publications:

Zalewski, A. A.: Effects of reinnervation on denervated skeletal muscle by axons of motor, sensory, and sympathetic neurons. *Am. J. Physiol.* 219: 1675-1679, 1970.

Zalewski, A. A.: Trophic influence of *in vivo* transplanted sensory neurons on taste buds. *Exptl. Neurol.* 29: 462-467, 1970.

Zalewski, A. A.: Prolonged survival and trophic function of neurons in *in vivo* homologous transplants of sensory ganglion. *Exptl. Neurol.* 30: (In Press)

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Neural Control, Intramural Research
National Institute of Neurological Diseases and Stroke

Karl Frank, Ph.D., Chief

Laboratory Objectives:

The objectives of the Laboratory of Neural Control continue to be:

1. The development of techniques for recording and analyzing the activity of the nervous system toward the ultimate goal of using this outward-directed information transfer for the control of external devices.

2. The development of techniques for delivering patterns of stimuli to the nervous system -- inward information transfer as in the case of a sensory prosthesis for the blind.

3. The study of basic mechanisms operating in the central nervous system by which it does its own controlling of motor behavior.

Staff:

Work toward achievement of these goals continues through six Laboratory projects and through the management of eight outside contracts. Nine budgeted positions are assigned to the Laboratory of Neural Control to carry out these functions. Other mechanisms bring the total number of persons in the Laboratory to 18. There are seven Civil Service employees; four Commissioned Officers of whom two are supported by other organizational units of NIH; two Visiting Fellows, one Special Fellow completing his second year, one Guest Worker, two temporary employees, and an electrical engineer who divides his time between our Laboratory and the Laboratory of Perinatal Physiology. Three of the staff members received promotions during FY 1971.

Of the nine budgeted positions assigned to the Laboratory of Neural Control, the equivalent of two full positions is devoted to the management of outside contracts, mostly for feasibility studies on a sensory prosthesis for the blind. An additional slot for a Secretary-Administrative Assistant is sorely needed to ease this load.

Two of our senior investigators have accepted positions at other institutions and both will continue the work they have been doing here. Thus, the total effort in the field of research related directly to neural control continues to increase. At the same time, these changes require some very important recruiting since the very nature of the Laboratory is determined by the research interests and efforts of its staff. A number of possible candidates are being considered for the open positions in an effort to find investigators whose primary interests lie in the application of techniques for recording from and stimulating chronically the central nervous system.

Techniques for Making Connections with the Nervous System:

The study of biologically acceptable materials for chronic implantation has continued both at the Laboratory of Neural Control and through feasibility studies of a sensory prosthesis for the blind. Percutaneous connectors of silastic with dacron skirts show promise and can be adapted to carry up to 50 leads.

Various techniques are being explored for recording from single neural elements in the CNS. These include both rigid and flexible microelectrodes and involve microcircuitry, RF sputtering, and electron beam evaporation. Telemetering devices are in use for transmitting neural information across the skin and electronic circuits have been devised for separating signals from different cells.

Motor Control Systems Operating in the Spinal Cord:

These studies describe: (1) the relationships between intrinsic properties of alpha motoneurons and physiological, morphological and histochemical characteristics of the muscle fibers they innervate; (2) the use patterns of different types of motor units; (3) the synaptic organization of motoneurons, and (4) the interaction of segmental and descending systems in control of information flow through the spinal motor mechanisms. The correlation of physiologically classified motor units with histochemically classified muscle fibers has been the result of a particularly effective collaboration between this Laboratory and the Medical Neurology Branch.

Experiments carried out in collaboration with the Surgical Neurology Branch have shown that total deafferentation in monkeys does not prevent the animal from learning to make precise movements of arm and hand, showing that sensory feedback from the limb is not essential for controlled motor output.

Cortical Control Mechanisms:

Neuronal spike trains from the motor cortex can be combined and processed so as to predict the time course of particular limb movements. New techniques have been utilized for computing linear (Wiener) filters which markedly enhance the correlation between spike trains of selected cells and a particular response measurement. A new servo mechanism has been devised for more precisely measuring and controlling the limb movements the monkey is trained to perform. This will permit further studies of the relationships between cortical cell firing patterns and the various measurable parameters of movement (force, position, velocity, etc.).

Electrophysiological Correlates of Learned Motor Acts:

Electrical patterns, both gross and single unit, of cortical neuronal activity have been found that are sufficiently well correlated with performance of learned (conditioned) motor acts, such as the eye blink, to provide a possible basis for identifying initiation of particular movements. Use of a matched filter with real neurophysiological data indicates that restricted motor behavior can sometimes be predicted under ideal conditions. The probabilities of detection and false alarm agree with theoretical calculations indicating that the matched filters being used approach optimal performance for detection of signals in real neuro-electric data.

In experiments using stimulation of the coronal-precruciate cortex, it has been shown that electrical thresholds are selectively lowered in areas whose stimulation leads to contraction of the target muscles of the conditioned motor response. Also, it has been shown that subliminal electrical stimulation of the cortex can be used as the conditioned stimulus for the development of a conditioned eye blink response.

Microstimulation of the Nervous System:

A new project has just been started in the Laboratory for studying the role of cells in the motor cortex in control of movement through the use of microstimulation techniques. Preliminary results indicate that conduction velocities of pyramidal cell axons in monkeys show a biomodal distribution over a sample of 250 cells from the pre-central gyrus, while 25 cells in the post-central gyrus show a unimodal distribution. No correlation has been seen between conduction velocity and depth of the cells in the cortex. In the future, activity of specific muscles will be monitored by EMG electrodes during stimulation of selected small groups of cortical cells for the purpose of investigating suprasegmental control of movement.

Sensory Prosthesis for the Blind:

Five contracts were in effect through FY 1971, with various institutions around the country for carrying out feasibility studies on development of a sensory prosthesis for the blind. The proposed device would operate through direct electrical stimulation of the visual cortex (or other parts of the visual system). Feasibility studies include stimulation of conscious human surgical patients to determine the nature of the subjective visual sensations elicited, and studies of the effects of implantation of various electrode material on the brain and on the implants themselves as a consequence of corrosive actions of the biological environment, particularly during chronic electrical stimulation over long periods.

Two new contracts have been negotiated to begin in FY 1972, both in the area of biomaterials. One of these is a study of the migration of implanted electrode materials into surrounding biological tissues and the changes which occur in the electrode surfaces. The other contract is for the study of the electrochemical reactions resulting when a metal electrode passes currents during stimulation. The practical goal of this contract is the elucidation of the optimal stimulus parameters for neural excitation with minimal biological or electrode damage.

While much remains to be learned about the patterns of stimulation necessary to elicit visual images in the blind, and no entirely satisfactory electrode system has been devised, still the possibility remains that a useful sensory aid may eventually be developed for reading and navigation.

Serial No. NDS(I)-70 LNLC/OC 1686
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Motor Control Systems in the Spinal Cord

Previous Serial Number: SAME

Principal Investigator: Robert E. Burke, M.D.

Other Investigators:
David N. Levine, M.D.
Felix E. Zajac, III, Ph.D.
Michael Salcman, M.D.
Peter Tsairis, M.D.
Ayub Ommaya, M.D.

Cooperating Units:
Medical Neurology Branch, NINDS
Surgical Neurology Branch, NINDS

Man Years:

Total:	3.1
Professional:	2.4
Other:	0.7

Project Description:

Objective: The objective of this project is to provide information in the following related areas: (a) concerning the interrelation between the intrinsic properties of alpha motoneurons and the physiological, morphological and histochemical characteristics of the muscle fibers innervated by the investigated motoneurons; (b) concerning the patterns of use of motor units of different type during a variety of movements in anesthetized and in alert, freely moving animals; (c) concerning the organization of synaptic input to alpha motoneurons from segmental reflex and descending systems; and (d) concerning the interaction of segmental and descending systems in the control of information flow in spinal segmental motor mechanisms.

Methods Employed: Anesthetized cats have been used in acute experiments involving intracellular microelectrode recording and stimulation techniques. Special methods have been developed for recording the mechanical responses of single motor units in cat hindlimb muscles following intracellular stimulation of their motoneurons. In collaboration with the Medical Neurology Branch, methods have been developed permitting histochemical identification of the muscle fibers belonging to motor units studied physiologically. We have also begun to explore a variety of techniques whereby the activity patterns of single motor units can be recorded in awake, freely moving animals and which permit subsequent physiological and histochemical characterization of the same motor units. Another aspect of the study of motor control mechanisms has involved examination of the effects of somatosensory deprivation on motor control. In collaboration with the Surgical Neurology Branch, monkeys have been prepared with posterior rhizotomy of cervical and thoracic roots, and these animals have been tested for motor control ability.

Major Findings: A. Physiological classification of motor units making up a mixed mammalian muscle. Building on earlier results in this Laboratory, we have re-evaluated the question of how motor units can be characterized according to their physiological properties. Surveys have been made of the entire population of motor units making up a mixed muscle (gastrocnemius) in the cat. This population of motor units can be divided into three basic types using a combination of physiological parameters, and such division includes all but a very few of the units present in this muscle. Examination of a relatively large number of physiologically definable parameters has permitted multivariable correlations which permit a rather comprehensive description of the motor unit population. A companion study has also been made of the relatively homogeneous soleus muscle in the cat, and the type of motor unit present in this muscle has been compared with the three types definable in the mixed gastrocnemius.

B. Histochemical characterization of the physiologically defined motor unit types. In collaboration with the Medical Neurology Branch, NINDS, we have been able to identify, in frozen sections of the whole muscle, the muscle fibers belonging to physiologically-studied motor units. This has permitted morphological and histochemical analysis of muscle fibers with measured physiological responses. The three physiological types of motor units present in the mixed gastrocnemius of the cat have been found to correspond with muscle fibers falling into three basic histochemical classes. The latter have been recognized for some time but the correlation of histochemistry with physiology has until recently been largely a matter of speculation. Comparison of the characteristics of gastrocnemius motor units with those present in the cat soleus muscle has led to the conclusion that the soleus motor units are in a number of ways different in both physiology and histochemistry from all three types present in the mixed gastrocnemius.

C. Activity patterns of single motor units of defined physiological type. Work has begun (spring 1971) on the problem of recording chronically from single motor units in cat leg muscles. Electrode design and radiotelemetry techniques have been adapted to this problem and data collection is under way.

D. Effect of posterior rhizotomy on motor control. After posterior rhizotomy of cervical dorsal roots, monkeys lose all sensory information from the forelimb. Nevertheless, such deafferented animals were found to be able to learn to exert precise levels of force in order to obtain food reward. Thus, immediate sensory feedback is not apparently necessary in this form of controlled movement.

Significance to Bio-Medical Research and the Program of the Institute: The problem of motor control in the intact animal involves consideration of the functioning of motor units as individual entities, active in asynchronous patterns. Much of the present work has been designed to further our understanding of the input-output relations of motor units, considered on the basis of single units rather than whole muscles or muscle nuclei. It can be expected that information regarding the organization of synaptic input to different groups of motor units will give a better picture of how the central nervous system may utilize these units during movements of various sorts. In addition, knowledge of the mechanical output characteristics of the same motor units permits inferences about the ultimate result of patterns of motor unit discharge in terms of muscle tension output. The motor unit has long been recognized as the ultimate target of integrative mechanisms in the motor system. The ultimate aim of this project is to contribute to a detailed understanding of the mechanisms, particularly those in and projecting to the spinal cord, which operate to control motor behavior.

Proposed Course of Project: During FY 1972, work on this project will include the following areas of concentration: (a) continuation of study of the activity patterns of single motor units in freely moving animals, together with an attempt to characterize the same units according to physiological and, hopefully, also histochemical type; (b) an assessment of the motoneuron intrinsic properties and synaptic organization of motor units in the three types now known to exist in the cat gastrocnemius muscle, basing this assessment on already existing data in these areas from earlier work in this Laboratory; (c) initiation of study of the peripheral and central effects of chronic overuse of a muscle, utilizing the techniques already developed and the information amassed about the normal situation.

Honors and Awards: None

Publications:

Burke, R.E., Jankowska, E., and ten Bruggencate, G.: A comparison of peripheral and rubrospinal synaptic input to slow and fast twitch motor units of triceps surae. J. Physiol. 207: 709-732, 1970.

Burke, R.E.: Control systems operating on spinal reflex mechanisms. Neurosciences Res. Prog. Bull. 9: 60-85, 1971.

Burke, R.E. and ten Bruggencate, G.: Electrotonic characteristics of alpha motoneurones of varying size. J. Physiol. 212: 1-20, 1971.

Burke, R.E., Fedina, L., and Lundberg, A.: Spatial synaptic distribution of recurrent and group Ia inhibitory systems in cat spinal motoneurones. J. Physiol. (in press)

Burke, R.E. and Nelson, P.G.: Accommodation to current ramps in motoneurons of fast and slow twitch motor units. Intern. J. Neuroscience. (in press)

Serial No. NDS(I)-70 LNLC/OC 1687
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Techniques for Making Connections with the Nervous System

Previous Serial Number: SAME

Principal Investigator: Frederick T. Hambrecht, M.D.

Other Investigators: Karl Frank, Ph.D.
Michael Salcman, M.D.
Edward M. Schmidt, Ph.D.

Cooperating Unit: Section on Technical Development

Man Years:

Total:	1.8
Professional:	1.3
Other:	0.5

Project Description:

Objectives: Each of the major goals of the Laboratory of Neural Control requires the development of techniques for making chronic connections to the nervous system for the purpose of selective recording and stimulation. Many parallel channels of information are required from individual nerve cells or small groups of cells of similar function. In accomplishing these objectives, it is necessary to: (a) develop new types of chronically implantable electrodes, (b) make connections between such electrodes and recording or stimulating devices outside the body, and (c) analyze the recordings from such electrodes so as to separate the signals from individual cells.

Methods Employed: A. Materials suited for biological implantation. Several materials have been chronically implanted into animals for evaluation of the stability of their physical properties and their biocompatibility. These materials include medical silastic 382, woven dacron, vitreous carbon, and a form of dental acrylic.

B. Percutaneous connections. Histological examination of teeth transplanted into the extended occipital prominence revealed dense fibrous adhesions between the teeth and the subcutaneous tissue. However, there was no indication of a gingival epithelial attachment nor an attachment of the alveolar bone to the bone of the calvarium.

Percutaneous connectors consisting of medical grade silastic with dacron skirts were fabricated. Three of these were implanted into a cat, and two are still intact after six months. A technique has been developed for easily inserting up to 50 leads through the connectors.

C. Designs for a chronic recording microelectrode. These studies have been concerned with the evaluation of systems (the electrode and its insulation, connectors and cables) which would permit chronic recording from small populations of separable neural elements, and the surgical procedures necessary in employing them. To date, we have investigated manual and mechanical means of electrode introduction; platinum-glass and platinum-quartz electrodes (tapered and unpolished, both) have also been studied.

Currently we are interested in the application of thin-film technology (microcircuitry, RF-sputtering, electron-beam evaporation, etc.) towards the production of a "tack" electrode, riding on a tether and affixed to the cortex by means of biological adhesives. The head of the "tack" will eventually contain a preamplifier.

Preliminary work with an older design permitted us to "hold" single units, in the awake animal, for as long as ten hours.

D. Evaluation of the use of telemetry for chronic recording of single cortical units and EMG in the cat. These studies have employed a miniature transmitter developed previously at NIH; means of implanting the unit in and on the animal, through the use of non-toxic "potting" materials, and a specially designed "saddle" (for mounting instruments and connectors), have been evaluated. Techniques such as these, together with a multi-stranded, stainless steel cable electrode, may enable us to follow the electrical activity of individual motor units in the freely moving animal.

E. Spike recognition. The spike recognition system described in last year's project report was designed to separate different waveshape spikes recorded from a single electrode. A modification of the instrument has been devised for use with multiple extracellular electrodes recording from peripheral nerve bundles. For spike separation of peripheral nerve traffic, both conduction velocity and spike shape are utilized.

Miniaturization of this spike recognizer is easily accomplished through integrated circuit techniques. Ultimately the system would be small enough that it could be carried by an implanted animal or man.

Major Findings: Silastic connectors appear to be accepted on a chronic basis by animals and should provide a means of reliable long-term percutaneous signal transfer.

Significance to Bio-Medical Research and the Program of the Institute: The mandate of the Laboratory of Neural Control includes the development of methods for direct control of external devices by the neural signals from the central nervous system. Other projects of the Laboratory explore the problems of what would be done with such signals if it were possible to record them chronically without damage. Thus, successful development of new techniques for stimulating and recording is mandatory for success of any of the Laboratory objectives. Such techniques will also be of broad general use in the basic neurophysiological research of other laboratories and branches.

Proposed Course of Project: Paragraphs B, C, and D under the section on "Methods Employed" describe techniques which will be further explored during the future course of this project. Developments covered in contracts on which Laboratory members are project officers are expected to contribute to some of these objectives as well as to the investigation of bio-materials described in paragraph A, "Methods Employed."

Honors and Awards: None

Publications:

Schmidt, E.M.: An instrument for separation of multiple-unit neuroelectric signals. IEEE Trans. Bio-Med. Eng. (in press)

Serial No. NDS(I)-70 LNLC/OC 1688
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cortical Mechanisms of Voluntary Motor Control

Previous Serial Number: SAME

Principal Investigator: Donald R. Humphrey, Ph.D.

Other Investigators: Karl Frank, Ph.D.
R. Gilbert Jost, M.D.
Edward M. Schmidt, Ph.D.

Cooperating Units: None

Man Years:

Total:	3.6
Professional:	2.5
Other:	1.1

Project Description:

Objectives: The major goal of this project is to quantify and study the relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. More specifically, we hope to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to: (a) estimate the time course and amplitude of various motor response measurements, and (b) determine which of a limited number of responses is occurring by observing only the firing patterns across the neuronal sample. If so, then such neuronal signals could conceivably be used for the control of prosthetic devices.

Methods Employed: Monkeys were trained to move a small handle by flexion-extension of the wrist, or by a simple push-pull motion involving rotation at the shoulder joint, against adjustable opposing forces. The position of the handle and the force exerted on it were continuously monitored; differentiation of these signals provided two additional output variables (velocity and dF/dt) with which unit activity could be correlated. Using a multiple microelectrode system, extracellular recordings were obtained simultaneously from several (4-8) selected neurons within the motor cortex of the unanesthetized animal as he performed the desired movements. Cells were selected on the basis of an apparent correlation between their firing patterns and variations in the response measurements, and were classified as pyramidal tract (PT) or non-PT on the basis of their response to stimulation of the medullary PT. A running count of the number of spikes per unit time (i.e., firing frequency) served as the measure of activity for each cell.

A new manipulandum has just been completed which is moved by a torque motor under servo control. As before, the force, position and velocity of the monkey's wrist movements are monitored. By operating the servo mechanism in various modes, all mechanical properties of the load (inertia, viscous friction, and spring constant) are under immediate control of the investigator and loads can suddenly be altered without warning while monitoring cortical cell firing and motor performance. It is hoped that by studying cortical cell and movement responses to such sudden load changes, more insight may be gained into the mechanisms of motor control.

Major Findings: As indicated in the last report, the neuronal spike trains could be passed through demodulating or smoothing filters, and the outputs combined by regression procedures, to obtain a surprisingly accurate estimate of the time course of the animal's net wrist torque or force output. These preliminary results were encouraging, but it was clear that the mathematical procedures were, for several reasons, non-optimal. Consequently, and in addition to gathering more experimental data, considerable effort has been devoted during the past year to setting up the necessary computer programs and exploring alternative quantitative methods for relating neuronal spike trains to various response measurements. The results of this exploration have extended our preliminary findings in two significant ways. First, with the use of systems analysis techniques, we have found it possible to compute linear (Wiener) filters which markedly enhance the correlations between the spike trains of selected cells and a particular response measurement. By computing the optimal low-pass filter for each unit, rather than arbitrarily setting its parameters as in previous experiments, a given level of prediction accuracy can thus be attained with the use of fewer simultaneously recorded spike trains. In addition, the accuracy is less affected by the loss of one of the spike train input signals than was the case with previous procedures. Secondly, and perhaps as important, these procedures can also be used to compute linear filters for relating the activity of cortical cells to the position and velocity of the animal's wrist. This finding modifies our previous conclusion, based on simple correlation techniques, that the activity of motor cortex cells appears only marginally related to limb position. Indeed, it suggests that such conclusions must always be interpreted in light of the quantitative techniques employed.

Significance to Bio-Medical Research and the Program of the Institute: Our additional analyses have provided further support for the concept that appreciable information about the animal's movement may be encoded in the temporal relationships between the spike trains of separate cells. Regardless of the quantitative procedures employed, prediction accuracies have invariably improved when these relationships were taken into account. Consequently, we feel that our results have important implications for current notions of output coding in the motor cortex. In addition, our findings suggest that if appropriate quantitative procedures are employed, it is possible to extract information about a number of motor performance parameters from the collective activity of a small group of selected cortical cells. Consequently, we are convinced that such signals could well be used for the control of prosthetic devices, should the problem of obtaining long-term recordings from a sufficient number of selected neurons eventually be solved.

Proposed Course of Project: The course of this project within LNLC will be interrupted due to the departure of the staff members involved with it, but it will be continued at the institutions to which they are moving. This is one of the most important projects within the mandate of the Laboratory, and it will be continued as soon as staff can be recruited whose interests lie in this area. Specific experiments will depend upon the principal investigator, but hopefully they will continue to explore methods by which neural activity recorded from the CNS can be used for the control of external devices, such as limb prostheses.

Honors and Awards: None

Publications:

Humphrey, D.R.: A chronically implantable multiple microelectrode system with independent control of electrode positions. Electroenceph. clin. Neurophysiol. 29: 616-620, 1970.

Humphrey, D.R., Schmidt, E.M., and Thompson, W.D.: Predicting measures of motor performance from multiple cortical spike trains. Science 170: 758-762, 1970.

Serial No. NDS(I)-70 LNLC/OC 1689
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrophysiological Correlates of Learned Motor Acts

Previous Serial Number: SAME

Principal Investigator: Charles D. Woody, M.D.

Other Investigators: Jerome Engel, Jr., M.D.
Mahmood Nahvi, Ph.D.
Henry R. Richardson, Ph.D.

Cooperating Unit: Daniel H. Wagner, Associates

Man Years:

Total:	2.4
Professional:	1.1
Other:	1.3

Project Description:

Objectives: Electrical patterns of cortical neural activity have been found that are sufficiently well correlated with the performance of learned motor acts to provide a possible basis for identifying the initiation of particular movements. Such patterns might provide a means for operating prosthetic devices where normal mechanisms of effecting neural control of movement are impaired. Present studies concern the assessment of different detection systems for recognizing such patterns and discriminating them from their associated background electrical activity. Other studies are directed to understanding how the organism produces a particular pattern of activity that can result in the initiation of a specific movement. Knowledge of the nature of the specificity of these electrical patterns in regard to motor performance and of the physiologic mechanisms that control the initiation of simple movements can facilitate the definition of patterns of electrical activity associated with more complex movements.

Methods Employed: Cats are trained to perform simple movements in response to natural or electrical conditional stimuli. Macroelectrodes are implanted chronically in motor areas of the cortex. Recordings are analyzed with a PDP-12 computer for patterns of activity correlated with the initiation of the learned movements.

Analytic techniques including adaptive filter operations developed by the principal investigator are used to determine the signal-to-noise ratio of the patterned electrical activity. Using matched filter detection techniques, percentages of successful detections are compared with error rate. Signal-to-noise ratio, successful detections, and errors are compared for theoretically optimal detection versus detection of the neurophysiological data. After training, microcurrent pulses are delivered to the motor region to establish the projection of the area studied and to determine the threshold of stimulation required to produce a particular facial movement. In separate studies, direct electrical stimulation of the coronal-precruciate cortex is paired with glabella tap. The electrical CS is delivered at an intensity which fails to produce a blink in the naive animal. Development of a conditioned motor response and the latency of the response following CS delivery are assessed in the animals after training.

Major Findings: A. The probabilities of detection and false alarm obtained from matched filtering of neurophysiological data could be predicted with reasonable accuracy from the theoretical operating characteristics of a receiver based on binary hypothesis test. Since a matched filter is an optimal linear filter for improving signal-to-noise ratio, the above finding suggests that we are approaching optimal performance for detection of the real neuro-electric data.

B. In cats trained to different motor responses, the threshold to electrical microstimulation is selectively lowered at areas of coronal-precruciate cortex that project ultimately to the target muscle of the conditioned motor response.

C. An electrical stimulus applied to a motor region of cat cortex is an adequate CS for development of a conditioned blink response. After training, stimuli which are sub-threshold for producing a response in the naive animal are able to elicit a conditioned blink movement. The latency of the conditioned response is 12 msec, that is, 4 msec longer than the 8 msec time required for conduction between coronal-precruciate cortex and the periphery. This conditioned response persists after ablation of cortical grey matter, bilaterally, posterior to the coronal-precruciate region. This and other evidence suggests that the coronal-precruciate cortex has the potential for undergoing a plastic, persistent change required for the development of learned motor performance.

Significance to Bio-Medical Research and the Program of the Institute: These investigations provide a theoretical basis for predicting successful detections versus errors for patterns of neuro-electric activity related to the initiation of movements. Such a theoretical basis, the applicability of which has been confirmed by experimental investigation, is required to assess the merit of using such patterns as the possible means for prosthetic control of movement. These investigations also suggest that control of the initiation of learned movements may depend upon the development of persistent changes in threshold in neuronal elements that project ultimately to the concerned muscle groups. It also appears that under certain circumstances classically afferent portions of the brain are not required for the initiation of learned movements.

Proposed Course of Project: This research will be continued by the principal investigator at the Mental Retardation Center, University of California at Los Angeles.

Honors and Awards: None

Publications:

Woody, C.D.: Conditioned eye blink: gross potential activity at coronal-precruciate cortex of the cat. J. Neurophysiol. 33: 838-850, 1970.

Woody, C.D., Vassilevsky, N.N., and Engel, J., Jr.: Conditioned eye blink: unit activity at coronal-precruciate cortex of the cat. J. Neurophysiol. 33: 851-864, 1970.

Woody, C.D., Engel, J., Jr., and Yarowsky, P.: Electrophysiologic evidence for a possible locus of a plastic change with conditioning. Fed. Proc. (in press)

Serial No. NDS(I)-71 LNLC/OC 1887
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Microstimulation of the Nervous System

Previous Serial Number: None

Principal Investigator: R. Gilbert Jost, M.D.

Other Investigators: Donald R. Humphrey, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: The aim of this project is to explore the potential for a relatively new technique, that of stimulating the brain through microelectrodes. Currently, very little is known about the type of pulse duration, current, waveshape, or size of electrode that is optimal for the excitation of a single brain cell, or a specific group of cells. Yet information of this kind is of vital importance to questions of neural control, as well as to our understanding of over-all neural function. It is clear that microstimulation offers a valuable technique for studying basic questions about the stimulation of the brain. In addition, the technique offers a promising means for studying the control of lower systems by higher centers. By exciting specific restricted areas of the brain, both the mechanisms as well as the means for controlling lower centers may be explored in new detail.

Methods Employed: Two independently movable microelectrodes are placed within a few microns of one another within the brain. With one electrode for stimulation and one for recording, it is possible to study in a very precise way the effects of stimulating a specific nerve cell under various stimulus conditions. In certain cases, when cells in the motor system are being studied, EMG electrodes are placed in appropriate muscles to explore the effect of this stimulation on motor control.

Major Findings: Our initial microstimulation studies are being carried out in the pyramidal motor system of the monkey. Information concerning the size and depth distributions of pyramidal tract cells has not been well worked out in this animal. Most of the basic work has been done in cats. Consequently, in order to form a better foundation for our microstimulation work, we have set out in our preliminary studies to investigate some basic physiological properties of the pyramidal system in the monkey. Recording from 250 pre-central pyramidal cells, we have found that the latency for antidromic conduction from the pyramid shows a bimodal distribution similar to that found in the cat. In the post-central region, however, data from 25 cells reveal that the latency distribution is unimodal, and all of the conduction velocities are rather slow (7-20 meters per sec.). This suggests a rather significant difference in the organization of the pyramidal tract between the cat and the monkey. We have found little or no correlation between latency and depth either pre- or post-centrally.

Significance to Bio-Medical Research and the Program of the Institute: Direct stimulation of the brain is a promising area for the field of neural control. Although current studies center on the motor system, information gained from this project will have important bearing on the feasibility of stimulating other areas of the brain. In addition, and perhaps no less important, studies of this kind inevitably lead to a better understanding of the underlying basis of how the nervous system works.

Proposed Course of Project: Our initial microstimulation experiments have centered on very basic questions concerning the parameters for stimulation of small areas of the brain. We expect to plot in detail the parameters necessary to stimulate cells of different sizes. In addition, we hope to arrive at some better hypotheses about the exact mechanism by which brain cells are stimulated. Information of this kind will then be used as a basis for more thoroughly investigating questions of suprasegmental motor control. Muscular activity in specific muscles will be monitored by strain guage and EMG while specific cells in the brain are stimulated with microelectrodes in an attempt to decode the relationships between higher and lower centers.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNLC/OC 1916
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Feasibility of a Sensory Prosthesis for the Blind

Previous Serial Number: None

Project Officers: Karl Frank, Ph.D.
Frederick T. Hambrecht, M.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Objectives: The possibility of using direct electrical stimulation of the central nervous system (CNS) in blind people as a form of information input for use as a sensory prosthesis is being investigated. Before the decision to construct and implement such a system is made, certain basic feasibility questions must be answered, such as the optimal stimulus parameters, the effects of chronic electrical stimulation on the CNS tissues, and the effects of the biological environment on the implanted system components.

Methods Employed: The feasibility studies are being investigated primarily through contracts with five institutions. The University of Utah has developed a "flying squad," which can be set up for electrical stimulation of the occipital cortex in any neurosurgery operating theater in the country on 24-hour notice. This group has also implanted, in animals subdurally and intracortically, over 40 different potential electrode materials to study their biocompatibility.

The Huntington Institute of Applied Research is developing a "drain" stimulator to be temporarily inserted as part of a Penrose drain in patients with chronic subdural hematomas. In addition, studies are under way on the effects of short-term and chronic electrical stimulation on the blood brain barrier (BBB) in animals.

The Massachusetts General Hospital will study human cases that require occipital craniotomies at their institution. They will focus on interaction of adjacent electrodes and effects of electrode size. They have also arranged a small sub-contract with Tufts Medical School for a neuroanatomical study of the horizontal intra-striatal connections in the Rhesus monkey.

The Johns Hopkins University has added stimulating electrodes along the shaft of their thalamic depth probe which is inserted in terminal patients for the relief of chronic pain. This allows stimulation of geniculo-calcarine tract as well as visual cortex. After autopsy, the exact position of the electrodes will be determined. The group is also studying the anatomic variability of the human striate cortex, the biocompatibility of potential materials, and the effects of chronic stimulation in animals.

The University of Rochester, using sighted and blinded Rhesus monkeys, is studying the long-term survival of electrical excitability, the possibility of producing seizure activity, the minimal electrode spacing which can still be resolved, the possible existence of a scanning mechanism for altering the excitability of striate cortex, and the existence of motor columns for eye movement in striate cortex.

In addition to the above-mentioned existing contracts, two new contracts have been negotiated to begin in FY 1972, for further studies in biomaterials. One of these will investigate the electrochemical reactions at the electrode-electrolyte junction during the passage of current. An attempt will be made to develop an effective electrode-stimulus current system which will minimize those electrochemical reactions that are detrimental to the tissue and to the electrode surface. The other contract involves a study of the surface erosion of potential electrode conductors, the migration of these conductors into tissue during chronic stimulation, and the investigation of novel conductors and insulators as potential electrode systems.

The Project Officers at NIH have performed safety experiments in animals in addition to those performed by the contractors. This has resulted in the establishment of stimulus parameter limitations for human experiments.

Major Findings: Twenty-one conscious patients have been studied acutely during craniotomies, two patients with drain stimulators have been studied, and five patients have had thalamic depth probes implanted. Appropriate electrical stimulation of the human striate cortex is usually reported by the patients as producing colorless, punctate spots of light (phosphenes) which flicker. Some of these patients had hemianopsias, and phosphenes were elicited in their blind visual fields. Early results indicate that the minimum electrode spacing for resolution of phosphenes is between 2-3 mm, and the intensity of the phosphenes can be controlled by varying the pulse amplitude, duration or frequency.

Stimulation of the geniculo-calcarine tract appears to produce more diffuse phosphenes which often contain patterns. Both this type of phosphene and phosphenes produced by cortical stimulation move with eye movement.

One of the most sensitive indicators of tissue damage is breakdown of the blood brain barrier. The use of direct-coupled pulses with a net direct current component produces severe tissue damage at current density levels which are effective for eliciting phosphenes.

Significance to Bio-Medical Research and the Program of the Institute: Should these studies indicate that a sensory prosthesis for the blind is feasible, the possibility of developing a truly useful sensory aid for reading and navigation would exist.

Proposed Course of Project: Further studies of the type indicated above are required. In addition, temporo-spatial pattern stimulation will be investigated.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Stroke

M.G.F. Fuortes, M.D., Chief

The physical facilities in the Laboratory of Neurophysiology have now been completed. A new Section on Neuronal Interactions has been added. The staff has been expanded and comprises now sixteen investigators, including four guest workers.

The research work performed during the year covers thirteen projects dealing with the physiology of photoreceptors, the organization of the retina, and the functions of the crystalline lens. Other major activities performed by the staff of the laboratory include the preparation of a Handbook of Sensory Physiology to be published by Springer-Verlag of Heidelberg, Germany; a series of lectures delivered to the medical students of Howard University; a lecture tour in the Soviet Union sponsored by the Exchange Program between the National Academy of Sciences and the Academy of Sciences, USSR; the organization of a course on photoreceptor physiology to be held in Erice, Italy, under the auspices of the "International School of Physics Ettore Majorana". The Exchange Program with the Academy of Sciences, USSR, will be supplemented during the fiscal year by the visit of a Soviet scientist who will perform experimental work in collaboration with the staff of the laboratory for a period of three months, starting in April 1971.

The research performed during the fiscal year divides into four main topics.

1) Studies on visual pigments. The molecular events leading to excitation of photoreceptors have been investigated in single cells by the use of microspectrophotometry, for which two new instruments have been developed. A dichroic microspectrophotometer is now being used for determining the absorption spectrum of single rods before and after bleaching. The spectra analyzed so far correspond well with those characterized in solutions for rhodopsin, meta rhodopsin I and II and retinal. The second microspectrophotometer is designed for rapid time resolution and will be applied to the study of chemical kinetics. The spectrographic studies give good promise to improve our understanding of the basic processes leading to sensory transduction.

2) Physiological studies of invertebrate photoreceptors. The electrical responses of visual cells in a number of lower

animals are analyzed with the purpose of establishing what mechanisms operate in the transformation of light into a neural response. Recordings from the leech photoreceptors show that the depolarization produced by light is due to an increase of conductance of the microvillar membrane. In addition, it is seen that the cell membrane reacts to depolarization with a graded response which causes delayed non-linearities of the current-voltage relations. The non-linear properties of photoreceptor membranes are being studied also in the cells of the ventral eye of Limulus, using voltage-clamp techniques. These studies are essential in order to resolve the problems raised in previous years when it was thought that the changes of membrane conductance evoked by light in photoreceptors are a consequence, rather than the cause, of the depolarization. Having identified many features of the depolarizing generator potentials of invertebrates, it became important to establish how neural interactions between receptors contribute to the processing of the information which receptor organs send to the central nervous system. This problem is being investigated both in the vertebrate retina (see section 3) and in invertebrates, where the analysis is easier due to the relative simplicity of the organization. Interaction studies are performed on the eye of the nudibranch mollusc Hermissenda which consists of only five photoreceptor cells. It is found that all cells are mutually inhibitory: as a consequence, activity in one will decrease (more or less effectively) the responses of all others. The signals which the five cells send to the central nervous system will then be determined by the light intensity falling on each cell, the sensitivity of the cells, and the inhibitory drive exerted on each cell by its neighbors. The possible role of these interactions in spatial and temporal discrimination is now under study.

It was seen last year that the ciliary photoreceptors of the mollusc Pecten are hyperpolarized by light. Since all microvillar photoreceptors investigated until last year are known to develop instead depolarizing potentials, it was thought that the sign of the potential change might be related to the evolutionary origin of the cell. Recent results are in disagreement with this simple interpretation since they have shown that the microvillar photoreceptors of the Salp (a primitive tunicate) are hyperpolarized by illumination. Hyperpolarizing responses are presumed to be evoked by light also in the photoreceptors of the clam, Mercenaria, because nerve impulses in this preparation are produced by dimming but not by raising light intensity. It has proved difficult, however, to confirm this supposition because the cell bodies of Mercenaria photoreceptors could not be penetrated by microelectrodes for intracellular recordings.

3) Organization of the turtle retina. The synapses between receptors bipolar and horizontal cells of the turtle have been

the subject of a major electromicroscopical study combined with Golgi staining. Two types of interactions have been demonstrated to occur in this retina which can be reasonably ascribed to these synapses: it has been shown that excitation of a receptor has a facilitatory effect on neighboring receptors, while excitation of horizontal cells exerts a delayed inhibitory reaction on the underlying receptors. This action of the horizontal cells extends over large areas of the retina and gives rise to peripheral inhibition of receptors: illumination of an annulus around a receptor decreases the hyperpolarization produced by the light applied directly on that receptor. Studies are now in course to determine what conductance changes are associated with the peripheral inhibition.

Recordings of horizontal and ganglion cell responses to different stimuli are also performed in order to establish how cellular interactions operate in organizing the signals produced by the retina.

4) Studies on the crystalline lens. Chemical agents have been found which influence the proliferation of the lens epithelium and the transparency of the lens itself. It is found that streptozotocin raises the blood sugar level, produces severe cataract, evokes retinal lesions and increases the incidence of renal adenomas. The lesions occurring in the retina and in the lens following administration of this agent are similar to those associated with diabetes in man. Study of the actions of this drug, therefore, may prove to be very useful for understanding the pathology of human cataracts and retinopathies.

Sixteen articles have been published this year by members of the staff to describe the results of the studies mentioned above.

Serial No.:NDS(I)-65 LNP/SP-1239
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Photoreceptors in the Limulus Eyes

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: George C. Murray, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	1.5
Other:	0.1

Project Description:

Objectives: The objective of this research is to gain insight into the means by which light is transduced into an electrical current in photoreceptors. The intracellularly recorded potentials of the photoreceptor cells of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods Employed: The responses of the photoreceptor cells to light and electrical stimulation are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. During this past year the technique of voltage clamping the membrane potential of Limulus ventral eye photoreceptors has been developed. The technique requires the penetration of a single photoreceptor with two microelectrodes, one for monitoring the membrane potential, the other for supplying the current necessary to clamp the membrane potential at the desired level. Using this technique, the role of visual pigments, of photoreceptor cell membrane and of various ions in photoreception can be studied.

Because of the necessity of taking many measurements from each experiment, a number of programs have been developed for the PDP-12 computer. With these programs and the PDP-12, both data accumulation and analysis has been made more efficient.

Major Findings: The major portion of the year has been spent in the development of the voltage clamp technique and of the computer programs mentioned in the Methods section. At present, therefore, our results must be considered preliminary. Our major findings are that the *Limulus* ventral eye photoreceptor membrane has voltage and time-dependent conductances and that the most striking effects of steady light stimuli are on the time-dependent components of these conductances.

Significance to Bio-medical Research and the Program of the Institute: Our preliminary results suggest that models of photoreceptor function based on linear and time-dependent components are inadequate to account for the properties of *Limulus* ventral eye photoreceptors and for the action of light on these cells. It is our hope that the results of our experiments will aid in the development of a more adequate model of photoreceptor function and in the understanding of the membrane characteristics of photoreceptors.

Proposed Course of the Project: It is proposed to continue the study of the membrane properties of *Limulus* photoreceptors by continuing the voltage-clamp techniques which have been developed and are now being applied to the study of membrane properties in light and darkness. The same method will also be extended to the investigation of the effects of altered ionic environment and of metabolic inhibitors on the functions of photoreceptors.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/SP-1690
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Rapid High Resolution Microspectrophotometry in Visual Cells

Previous Serial Number: Same

Principal Investigator: George C. Murray, Ph.D.

Other Investigators: Thomas G. Smith, Jr., M.D.
Theodore Colburn, Ph.D.[1]

Cooperating Units: [1]Section on Technical Development, NIMH-NINDS

Man Years:

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives: The objective of this research is the instrumentation and use of rapid scanning microspectrophotometric techniques for the in situ investigation of the chemical kinetics within single excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: A new microspectrophotometer has been designed which can sample transmittance at spectral wave bands between 350 and 600nm, either sequentially or in random order, at rates of 10 microsecond per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by a cathode ray tube face. By moving the spot on the face appropriately, the spectral output of the monochromator is changed. The beam intensity can be rapidly controlled to allow 1) modulation at high frequency and the use of coherent detection of the transmitted light and 2) precise control of the average intensity during a sampling period. In the future, the use of an on-line computer will provide simplified program control and analysis of the complex spectral changes to reveal the kinetics of the underlying chemistry.

Major Findings: The prototype system has been constructed and used to develop the system concepts and circuits. The analysis electronics and much of the control circuitry has been designed and constructed and preliminary system tests made. These have shown the basic system to be functional but has pointed out a need for further engineering work on the CRT system which currently suffers from insufficient position stability and intensity. The positioning circuitry is currently being worked on. The other shortcoming, a lower than anticipated signal to noise in the measured photocurrent, is primarily due to a failure to achieve hoped for phosphor light output. Current state of the art will allow improvement in signal to noise only by a sacrifice in speed of about a factor of 8-10.

Significance to Bio-medical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: We propose to continue the construction and testing of the rapid microspectrophotometer system, while the electrophysiological properties of the photoreceptor membranes are being better quantified as discussed in report NDS(I)-65 LNP/SP-1239. We will also investigate the extension of fast photometric techniques to the investigation of other intracellular systems.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/CB-1659
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Synaptic Contacts of Visual Cells

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: Paul M. O'Bryan, Ph.D.
M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	1.0
Other:	0.7

Project Description:

Objectives: To investigate the fine structure and function of the synaptic contacts of retinal visual cells.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi. Intracellular microelectrode recordings of the electrical activity of retinal cells.

Major Findings: Electron microscopic work on the turtle (Pseudemys scripta elegans) retina has been continued to try to establish the structure of the end contacts of basal processes of the cone pedicles. Golgi techniques combined with electron microscopy show that the basal processes end in contact with horizontal cell processes lodged within the pedicles of other cone cells. The fixation obtained with this method, however, was too poor to determine the structural features of these contacts. Serial sectioning has been, therefore, attempted as an alternative approach to this problem, but as yet without success. This is because the structural analysis of the contacts requires pictures at relatively high magnification, while the length of the basal processes (10-40μ) makes it necessary to follow them in low power serial pictures covering large fields.

The morphological work on the turtle retina are now being followed by electrophysiological studies on synaptic interactions between receptor cells and between them and second order neurons. Excitatory and inhibitory synaptic inputs on turtle cones have already been found in this laboratory by Baylor, Fuortes, and O'Bryan (J.Physiol, in press). These observations are now being extended by an analysis of the effect of a large peripheral field on the cone cells. By a suitable choice of stimulation parameters, it has been found possible to enhance such effect, so that its properties can be studied in more detail. The peripheral response consists of a depolarization superimposed on the hyperpolarization induced by light falling directly on the cone cells. Since this antagonism between center and periphery resembles that found by other workers at the bipolar cell level, it seemed desirable to ascertain -- by means of dye injections -- the identity of the cells giving the enhanced peripheral response. In all instances, they were found to be cone cells, as already reported by Baylor et.al, for the more subtle inhibitory effects observed previously.

The mechanism of origin of the peripheral inhibition of cone cells is now under study. It appears to be associated with a composite conductance change involving more than one ionic mechanism, but it is as yet unclear whether more than one synapse is responsible for the observed changes. From the electron microscopic evidence, it would seem possible that horizontal cells, as well as some types of bipolar cells, may feed back on the cone pedicles.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: The study of the conductance changes associated with the peripheral inhibition of cone cells will be continued, and attempts will be made to establish whether separate synaptic pathways are involved in the genesis of the individual components of these conductance changes. Electron microscopic studies of the turtle retina will be continued in other species in the hope of finding instances where the synaptic connections of the cone cells lack the complexity found in *Pseudemys*.

Honors and Awards: None

Publications:

Lasansky, A.: Synaptic organization of cone cells in the turtle retina. Philosophical transactions of the Royal Society. London. Series B. (In press).

Fuortes, M.G.F.: Generation of responses in receptors. In Loewenstein, W.R. (Ed.): Handbook of Sensory Physiology. Heidelberg, Germany, Springer-Verlag, 1971, Vol. 1, pp. 243-268.

Serial No.:NDS(I)-69 LNP/CB-1692
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Responses of Retinal Cells in Vertebrates

Previous Serial Number: NDS(I)-69 LNP/GP-1692

Principal Investigator: Paul M. O'Bryan, Ph.D.

Other Investigators: Arnaldo Lasansky, M.D.
M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Objectives: This research project deals with the mechanisms by which electrical signals are generated and transmitted by cells in the vertebrate retina in response to light stimuli. To date attention has been focused on the receptors, the first order cell in the visual pathway and the horizontal cells which are second order cells, being synaptically activated by receptor activity.

Methods Employed: The preparation used is the retina of the turtle (Pseudemys elegans) which was chosen because of the relatively large size of the cells and the cellular architecture appeared rather simple. Microelectrodes are used to record the activity of single cells during stimulation of the retina with light. Studies on the fine structure of the turtle retina has been carried out by one of the investigators.

Major Findings: It was previously noted that the electrical responses of single cones depended not only on the light directly impinging on the receptor but also on the area of illumination, i.e., stimulation of activity in surrounding receptors synaptically modified the response of a central receptor. Two effects of illumination of the periphery of a receptor were noted:

1) illumination of the near surround (spots $\leq$ 50) resulted in facilitation of the receptor response to light and, 2) illumination of the far periphery (spots $\leq$ 150µ) resulted in a delayed inhibition of the receptor response which tended to shorten the response of the receptor to light. These findings suggested that interactions at the level of the receptors occur which are mediated by two cell types having different receptive fields.

To demonstrate which cells were mediating the two effects, simultaneous recordings from two retinal cells were made. One of the cells penetrated was polarized by passing a current through the recording electrode to determine if a potential change in this cell could affect the potential of the receptor being recorded from. It was found that if two receptors were penetrated which were within 50µ of each other, that coupling between these cells could be demonstrated;if the receptors penetrated were separated by more than 50µ no coupling existed. This result is consistent with the idea that the facilitation of the receptor response is due to direct receptor coupling. On the other hand, hyperpolarization of a horizontal cell produced a depolarizing synaptic potential in a receptor which inhibited the direct response of the receptor to light. This finding demonstrates that horizontal cells mediate an inhibitory feedback onto receptor cells and are responsible for the inhibitory effect produced by illuminating the far periphery of the receptor.

Experiments are now in progress to determine the mechanism responsible for the horizontal cell mediated inhibition of the receptor response. Conductance measurements of the inhibitory potential change indicate that it is made up of two components, (i.e., the opening of two separate conductance channels having different reversal potentials). This suggests that the feedback onto receptors may be mediated by two different cells or that the transmitter released has a byphasic action on the postsynaptic receptor membrane.

Significance to Bio-medical Research and the Program of the Institute: One of the largest gaps in our understanding of visual physiology is how the signals in retinal cells are generated. These results and their interpretation help to understand how the signals are generated and how one cell in the retina influences the activity of other retinal cells.

Proposed Course of the Project: We propose to study further the nature of the conductance changes producing the inhibition of the receptor response. In addition, since the coupling between receptors within 50µ is not always present, this suggests that receptor coupling may be specific as for example only between cones sensitive to the same wavelength of light. We plan to examine this possibility and to study the properties of receptor coupling more carefully.

Honors and Awards: None

Publications:

Baylor, D.A., Fuortes, M.G.F., and O'Bryan, P.M.: Receptive fields of cones in the retina of the turtle. J. Physiol. 214: 265-294, 1971.

Baylor, D.A., and O'Bryan, P.M.: Electrical signaling in vertebrate photoreceptors. Fed. Proc. 30: 79, 1971.

Fuortes, M.G.F., and O'Bryan, P.M.: Generator potentials in invertebrate photoreceptors. In Fuortes, M.G.F. (Ed.): Handbook of Sensory Physiology. Heidelberg, Germany, Springer-Verlag, 1971, Vol. VII/2, Chapt. 6. (In press).

Fuortes, M.G.F., and O'Bryan, P.M.: Responses to single photons. In Fuortes, M.G.F. (Ed.): Handbook of Sensory Physiology. Heidelberg, Germany, Springer-Verlag, 1971, Vol. VII/2, Chapt. 7. (In press).

Serial No.:NDS(I)-71 LNP/GP-1888
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Eye Changes in Prolonged Streptozotocin-induced Diabetes in Rats

Previous Serial Number: None

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.4
Other:	0.1

Project Description:

Objectives: To explore the long-term ocular and systemic effects of streptozotocin-induced diabetes in rats as a new experimental model of the human disease.

Methods Employed: Ten Osborne-Mendel male rats were injected with streptozotocin (65 mg/kg i.p.) at six weeks of age. The course of the resulting hyperglycemia was followed by regular determinations of non-fasting blood glucose levels and the development of ocular lesions by frequent slit-lamp examinations. It was planned to maintain these rats together with untreated control animals for at least one year to observe any slowly-developing signs of damage particularly in retina and kidney. Four rats died during this period and one had to be killed at 9-1/2 months because of debilitation. The remaining five animals were killed one year after administration of streptozotocin. Complete autopsies were performed and the eyes, pancreas, and kidneys were routinely prepared for histological examination.

Major Findings: The histological picture of the long-standing total cataracts present in all diabetic animals was similar to that reported earlier. Unfortunately, the presence or absence of diabetic lesions in the retina could not be determined due to the development of a severe retinal degeneration in all rats over 9 months of age in both the experimental and control groups. This condition appeared to be hereditary and was not related to the diabetic state.

In an earlier series of streptozotocin-treated rats, we had observed benign renal adenomas in all of three animals killed between seven and nine months. In the present group of rats, large adenomas occurred in all kidneys of the six rats surviving to autopsy. Multi-focal proliferation of the tubular epithelium gave rise to solid or cystic adenomas varying in size from less than a millimeter to as much as 3 centimeters in diameter. The kidney was the only organ that showed neoplasia on gross and histopathologic examination. No tumors developed in control animals.

Evaluation of glomerular changes in the diabetic rats is in progress and no conclusion can be drawn at this time.

Significance to Bio-medical Research and the Program of the Institute: Study of streptozotocin diabetes as a new model of the human disease may provide further information on the complex pathological processes in man. The tumorogenic property of streptozotocin is of particular interest in view of the use of the drug in the chemotherapy of islet cell tumors.

Proposed Course of the Project: Since vascular lesions in the retina could not be identified because of the spontaneous retinal degeneration appearing in this strain of rats, experiments with other strains are considered. The retinal lesion observed in dogs with alloxan diabetes resembles diabetic retinopathy in humans, and encourages further long term studies on experimental diabetes.

Honors and Awards: None

Publications:

von Sallmann, L., and Grimes, P.: Histopathologic Changes in the Eyes and Kidneys of Rats with Long Term Streptozotocin Diabetes. Current Classics in Ophthalmology. (In press).

Serial No.:NDS(I)-71 LNP/GP-1889
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on agents affecting cell proliferation and differentiation in the crystalline lens

Previous Serial Number: None

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	1.6
Other:	0.1

Project Description:

Objectives: Among agents which stimulate cell division, isoproterenol (IPR) is known to induce a rapid increase in DNA synthesis and mitosis in the salivary glands of mice and rats. The drug appears to affect the salivary gland cell directly, through a beta-adrenoreceptor system, but the mechanism remains obscure. We have observed the effects of both systemic and local administration of IPR in the lens epithelium of rats to determine the action of the drug in a non-innervated population with a well defined cell cycle.

Methods Employed: Young Osborne-Mendel female rats were treated with IPR in a dose sufficient to elicit marked stimulation of salivary gland cell proliferation (0.8μM/g body weight i.p.). In other groups of animals the drug was administered locally by sub-conjunctival injection or instillation of drops in one eye only. The animals were killed at specific intervals between 4 and 48 hours after drug injection. The total number of mitoses in whole flat mounts of lens epithelium were counted and compared with control values. In some experiments, the rats were injected i.p. with H^3-thymidine one hour prior to death and the number of cells in DNA synthesis was established from counts of labeled nuclei in autoradiographs.

Major Findings: Intraperitoneal injection of IPR (0.8µM/gm body weight reduces mitotic activity in all zones of the epithelium to approximately 35% of normal values at 24 hours. This inhibition is followed by a secondary stimulation of cell division at 48 hours. On the basis of total mitosic count per preparation, the increase at this interval amounts to only 40% above control counts. However, when the three zones of the epithelium are compared, it can be seen that nearly all of the increased activity occurs in the equatorial region where mitoses are almost twice as frequent as in untreated animals.

Rats treated with this large dose of IPR are in severe distress for several hours. Although there is neither pupillary dilatation nor cataract formation during this period, some widening of the lid fissure is often seen. These observations suggested that the mitotic changes may occur secondarily to alteration of the lens environment due to systemic effects or exposure of the cornea.

In a small group of rats one eye was closed by suturing the lids prior to drug adminstration to eliminate the possibility of changes in aqueous humor composition due to evaporation from the exposed cornea. Under these conditions, no significant difference in mitotic counts between open and closed eyes of the same animal could be demonstrated at 24 hours.

To reduce possible systemic effects, the drug was applied locally either by instillation or sub-conjunctival injection. Instillation of one drop of a 5% solution of IPR each hour for 8 hours did not cause dilation of the pupil in the treated eye but the animals showed characteristic signs of distress due to systemic absorption of the drug. Inhibition of mitosis was apparent in both eyes of these animals at 24 hours although the treated eye was more affected.

Sub-conjunctival injection of IPR was found to be a more efficient method of local application. Doses of 0.1 and 0.05 µg/gm body weight caused maximum pupillary dilatation and transient lens opacities in the treated eyes. Lower doses of IPR, 0.025, 0.010, and 0.005 µM/gm body weight, caused moderate to minimal pupillary dilatation but no lens opacification. At the two lowest doses mitotic activity was inhibited by about 50% in the treated eyes at 24 hours with no significant effect in the untreated eyes.

Significance to Bio-medical Research and the Program of the Institute: The clarification of the effects of IPR on this non-vascularized, non-innervated epithelium may contribute to the understanding of the complex mechanisms whereby such agents act on cell populations of different functional properties.

Proposed Course of the Project: After the studies on DNA synthesis are completed, IPR will be tested in organ culture of the lens in an effort to distinguish between direct and indirect effects of the drug.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/GP-1890
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Organization of a Molluscan Visual System

Previous Serial Number: None

Principal Investigators: Daniel L. Alkon, M.D.
M.G.F. Fuortes, M.D.

Other Investigators: Leona M. Stewart

Cooperating Units: None

Man Years:

Total: 2.0
Professional: 1.6
Other: 0.4

Project Description:

Objectives: The principal objective is to understand how information provided by light stimuli is processed in an eye containing only five cells. The experiments are performed on Hermissenda Crassicornis, a nudibrand mollusc. Work planned will concern, in addition, a fourteen cell optic ganglion, believed to contain second order neurons, as well as higher-order cells in the brain.

Methods Employed: The visual system of Hermissenda Crassicornis was chosen because of its relative simplicity. The eye consists of five cells whose axons extend through a small ganglion of fourteen cells. Intracellular recording from single receptor cells as well as receptor cell pairs has been the principal experimental technique used thus far. Iontophoretic injection of a fluorescent dye, Procion yellow, is also being used to study the anatomy of the system.

Major Findings: All five receptor cells give a depolarizing response to light. The magnitude of depolarization as well as the frequency of impulse firing is graded with the intensity of the light stimulus. Data collected so far indicate the presence of two cells with large spikes, high threshold of firing to depolarizing current and pronounced after-hyperpolarization following a bright flash. The three remaining cells have smaller spikes (<25m), lower threshold of firing to depolarizing current, and a high frequency of after-firing following a bright flash. In all cells, the responses to bright flashes may include oscillations. In different cell pairs these may be in phase or out of phase. All of the cells appear to be mutually inhibitory.

Work has also been done to characterize conductance changes of the cells during illumination as well as the chromatic sensitivity at the cells. The Dartnall nomogram for rhodopsin fits well the action spectrum obtained for the visual cells studied in our preparation.

Significance to Bio-medical Research and the Program of the Institute: The visual system of Hermissenda Crassicornis may be the first preparation where careful definition of a neural network is possible. Such definition might contribute to our understanding of the visual process as well as to neural organization in general.

Proposed Course of the Project: Once the interconnections of the five visual cells have been established, experiments are planned to test the usefulness of these connections for processing visual information. Intracellular recording from second and third order cells would then follow. Anatomic description of both the receptor cells as well as the second order cells will be attempted first by Procion injection and possibly later with other histologic techniques.

Honors and Awards: None

Publications:

Fuortes, M.G.F.: Responses of photoreceptors. Psychiatric Research. (In press).

Serial No.:NDS(I)-71 LNP/GP-1891
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Origin of Electrical Response in Photoreceptor Cells

Previous Serial Number: None

Principal Investigators: Roberto Fioravanti, M.D.
M.G.F. Fuortes, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.7
Other:	0.1

Project Description:

Objectives: To investigate the location of the excitable membrane and the nature of the transducing mechanism in visual cells.

Methods Employed: Electrophysiological recordings.

Major Findings: In this work the resistances and time constants in leech visual cells are measured. Taking advantage of the peculiar structure of the leech visual cells, records are taken from the cytoplasm and also from the external fluid immediately adjacent to the microvilli. In this way, it should be possible to measure both the potential changes and the membrane currents evoked by illumination. The measurements indicate that the response to a step of current is a linear combination of two exponentials. Studying the features of the responses to currents in dark, and during constant illumination, as well as the responses to brief pulses of light, we are searching for the best and simplest model which fits the data. Measurements from the cytoplasm show that the membrane has a very high resistance in dark (100MΩ) and a much lower resistance in light (20MΩ). The time constants are about 5msec and 50msec in darkness. Following illumination the slow component can disappear almost completely while the fast component does not change greatly.

Significance to Bio-medical Research and the Program of the Institute: These results provide a better understanding of the processes mediating visual excitation.

Proposed Course of the Project: The work will be continued until sufficient measurements are obtained, to determine what changes are produced by light in resistance, capacity and electromotive forces of the microvillar and of the external membrane of the cell.

Honors and Awards: None

Publications:

Fuortes, M.G.F.: The effect of cell volume on the visual responses of the eye of Limulus. In: Liber Memorialis, in honor of Dr. Chandler Mc C. Brooks. (In press).

Serial No.:NDS(I)-71 LNP/GP-1892
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparative Physiology of Invertebrate Photoreceptors

Previous Serial Number: None

Principal Investigator: John S. McReynolds, M.D.

Other Investigators: Anthony L.F. Gorman, Ph.D./1

Cooperating Units: 1/St. Elizabeth's Hospital

Man Years:

Total:	1.3
Professional:	1.2
Other:	0.1

Project Description:

Objectives: To study the mechanisms by which light energy is transformed by photoreceptors into electrical signals, and how this information is transmitted to higher-order cells in the nervous system. In particular, this is a comparative study of the ways in which different types of photoreceptors generate and use both depolarizing and hyperpolarizing responses to light.

Methods Employed: This project has begun to study possible correlations between structure and function in several types of invertebrate eyes, using both electron microscopy and intracellular recording with microelectrodes. Studies have been done on the photoreceptors of four species: Amaroucium constellatum (phylum chordata); Salpa democratica (phylum chordata); Lima scabra (phylum mollusca); Pecten irradians (phylum mollusca).

Major Findings: Most invertebrate photoreceptors have microvillous membrane specializations and depolarizing receptor potentials, whereas those of vertebrates have photoreceptive areas derived from Cilia, and hyperpolarizing receptor potentials. It has been shown that the ciliary-type photoreceptors in an invertebrate, Pecten irradians, also hyperpolarize. However, although the hyperpolarization of vertebrate photoreceptors is associated with a decrease in membrane conductance, that of the invertebrate Pecten, like the depolarizing of other invertebrate receptors, is associated with an increase in conductance. To determine whether any of these apparent correlations have a functional significance, we have investigated these properties in visual cells of some primitive members of the same phylum as vertebrates, which might be expected to have features common to both groups.

The photoreceptors of the larval tunicate Amaroucium constellatum have whorls of flattened membranes which are derived from a ciliary base and may be regarded as modified cilia. Electrophysiological recordings show that these cells are hyperpolarized by light and the response is associated with a decrease in membrane conductance. In another tunicate, Salpa democratica, the photoreceptor cells have microvilli, and no evidence of cilia. These receptors respond to light with a hyperpolarizing receptor potential and an increase in membrane conductance.

These results indicate that there is no essential correlation between the fine structure of the photoreceptive membrane and the polarity of the response to light. It appears that the evolution of chordate photoreceptors (including vertebrates) has led to a hyperpolarizing receptor potential, but in different species this has been achieved by utilizing both ciliary and microvillous membrane specializations, and both increases and decreases in membrane conductance. Experiments are also being conducted to study the way in which the different types of receptor response are used to transmit information. These studies involve another mollusc, Lima scabra, as well as the above mentioned animals. In Lima, as in Pecten, there are ciliary-type, hyperpolarizing receptors which transmit information by action potentials fired at the cessation of illumination. In Salpa, however, it appears that information is transmitted during the hyperpolarizing receptor potential since higher order cells also respond during illumination. In this respect the Salp photoreceptors resemble those of vertebrates, although the conductance change underlying the hyperpolarizing receptor potential is in the opposite direction. In Lima, current studies are focused on the mechanism of production of the "off" response following illumination. This "off" response may be simply a result of the previous hyperpolarization during illumination. However, other possibilities which may contribute, and which have not yet been ruled out are 1) a separate slower photochemical process which generates the "off" response after the hyperpolarization, 2) a simple change of concentration gradients during the response, 3) activation of an electrogenic pumping mechanism consequent to the change in some ionic concentration during the hyperpolarizing response.

Significance to Bio-medical Research and the Program of the Institute: One of the largest and most important gaps in our understanding of visual physiology concerns the basic mechanisms of receptor signaling. The results and their interpretation help to further our knowledge of how signals are generated and transmitted by the receptors.

Proposed Course of the Project: In the Salp photoreceptor; 1) How the cell is able to produce very large responses (70mV) with a resting potential of only about 10mV. What ions determine the resting potentials and the response to light?

2) The nature of the extremely slow time course of the response to light. This receptor appears to be able to integrate the quantity of weak light over many seconds.

3) How is information transmitted to higher order cells? Does the hyperpolarization cause release of a transmitter substance, or does it turn off a transmitter which is released in darkness?

In the Lima photoreceptor, we will further study the mechanisms of production of the "off" response in the hyperpolarizing photoreceptor.

Honors and Awards: None

Publications:

McReynolds, J.S., and Gorman, A.L.F.: Photoreceptor potentials of opposite polarity in the eye of the scallop, Pecten irradians. J. Gen. Physiol. 56: 376-391, 1970b.

McReynolds, J.S., and Gorman, A.L.F.: Membrane conductances and spectral sensitivities of Pecten photoreceptors. J. Gen. Physiol. 56: 392-406, 1970b.

Barnes, S.N., Gorman, A.L.F., and McReynolds, J.S.: Fine structure and intracellular responses of photoreceptors of a pelagic tunicate, Salpa. Biol. Bull. 139: 414, 1970.

Gorman, A.L.F., McReynolds, J.S., and Barnes, S.N.: Photoreceptors in primitive chordates: fine structure, hyperpolarizing receptor potentials, and evolution. Science. 1971. (In press).

Serial No.:NDS(I)-71 LNP/GP-1893
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neural Organization of Photosensory Systems

Previous Serial Number: None

Principal Investigator: Eric A. Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description:

Objectives: The program is designed to elucidate examples of functional organization of photosensory systems.

Methods Employed: Current techniques of intracellular and extracellular recording from single neurons and of intracellular dye iontophoresis are utilized.

Major Findings: Investigation has proceeded in two areas. Initially the compound eye of Limulus has been studied as a model of lateral inhibitory interaction. It was determined by intracellular fluorescent dye iontophoresis that eccentric cells do and retinular cells do not contribute to the anatomic lateral plexus. Subsequently it was determined that although both cell types send axons into the optic nerve, only eccentric cells generate propagating impulses to light stimulation. It was therefore concluded that the lateral inhibitory model represents a mode of interaction between eccentric cells only.

Later investigation dealt with the organization of vertebrate retinal ganglion cells. Several cell types in the turtle retina have been characterized in terms of receptive field and superthreshold response.

Significance to Bio-medical Research and the Program of the Institute: The work should contribute to our understanding of the principles of anatomic and functional organization of the nervous system and of the retina in particular. Investigation of the Limulus compound eye has contributed to our understanding of this invertebrate system. Similar forms of interaction may be in part the basis of more complex systems.

Proposed Course of the Project: It is hoped to determine the contribution of the several cell types in the retina to ganglion cell response and the rules for interaction between these sets.

Honors and Awards: None

Publications:

Schwartz, E.A.: Retinular and Eccentric Cell Morphology in the Neural Plexes of *Limulus* Lateral Eye. *J. Neurobiol.* 2: 129, 1971.

Serial No.:NDS(I)-71 LNP/NI-1894
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Synaptic transmission between photoreceptors and horizontal cells in the turtle retina

Previous Serial Number: None

Principal Investigator: Luigi Cervetto, M.D.

Other Investigators: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.4
Other:	0.1

Project Description:

Objectives: The objective of this research is to study the mechanism of excitation and conduction between photoreceptors and horizontal cells.

Methods Employed: Electrical responses to light have been recorded from horizontal cells in the turtle retina. Short pulses of radially directed current were applied across the entire retina. The intracellular behavior of the horizontal cells to the radial polarization has been analyzed.

Major Findings: It has been observed that sclera-positive pulses of current produce a transient depolarization of the horizontal cell. The amplitude of this depolarization depends upon the intensity of the light stimulation.

In general, the amplitude of the depolarizing response produced by current increases as far as the response of horizontal cell to the light stimulation increases. When the amplitude of horizontal cell response reaches the saturation level, further increases in light intensity produce a decrease of the transient depolarization yielded by current.

Pulses of current of opposite polarity (vitreous-positive) are generally ineffective and very seldom produce a small depolarizing response.

In the chromatic units in which the polarity of the response depends upon the wavelength of the light stimulus, the effect of short pulses of current is depolarizing when the light produces a hyperpolarizing response. When the light produces a depolarizing response in the horizontal cell, the effect of current becomes hyperpolarizing.

Significance to Bio-medical Research and the Program of the Institute: This project should provide some information about the function of the synaptic connections between receptor and horizontal cells.

If the effect produced by current is mostly due to the polarization of the receptor, the present findings would suggest that the depolarization of the receptor endings is effective in releasing a transmitter whose effect is to depolarize the horizontal cell.

Proposed Course of the Project: The results of this study cannot be meaningfully interpreted until more information is obtained about the effect of current upon the receptor.

The present approach will be continued and the effect of extrinsic current on the receptors will be studied. By using a perfused isolated retina, the possibility of affecting the receptor-horizontal cell synapses with pharmacological agents will also be investigated.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/NI-1895
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Visual Pigments Situated in Single Receptors of the Frog Retina

Previous Serial Number: None

Principal Investigator: Ferenc I. Harosi

Other Investigators: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	1.5
Other:	0.1

Project Description:

Objectives: The project is aimed at gaining insight into the molecular events associated with visual excitation in the photoreceptors of a vertebrate.

Methods Employed: The principal tool of this investigation is single-cell microspectrophotometry. A novel instrument was designed and built which has been called the dichroic microspectrophotometer (DMSP). This DMSP, by measuring the transverse dichroic absorption of the outer segment of a rod or cone cell, allows one to follow orientational and spectral changes occurring in visual pigment chromophores subsequent to light absorption. Due to its rapid operation (a complete spectrum is scanned in 3/4 of a second), the DMSP provides us with a temporal resolution of a few seconds; this feature is made use of in detecting some relatively long-lived intermediates of the bleaching process.

Major Findings: Preliminary measurements on single frog rod outer segments indicate that the following are true: a) high transverse absorption (24% per cell diameter); b) high dichroic ratio (between 7 and 8); c) extremely good agreement in wavelength of peak absorption (502 nm) and shape of the absorption spectrum (in the range of 400 to 695 nm) as measured in a single rod as compared with Dartnall's data (1953) obtained by solution photometry of digitonin extracted rhodopsin; d) while absorption (about 20% of that at 502 nm) and dichroism persist in the u.v. portion of the spectrum (325-400 nm), the β-band appears to be lacking; e) the first bleaching intermediate appears to be meta I (at 22°C and pH 6.8), as resolved by the DMSP. The evidence for this labile intermediate to be meta I is that (1) its chromophores are transversely oriented just as the native rhodopsin chromophores are, and (2) that its absorption peaks at 480 nm, and (3) that the shape of its absorption spectrum follows closely a hypothetical pigment characteristic of 480 nm maximum derived from the rhodopsin based Dartnall nomogram; f) the second intermediate to be detected is meta II, absorbing maximally at 380 nm, with still transversely oriented chromophores; g) simultaneously to meta I and meta II, retinal appears in the cell; it also absorbs maximally at 380 nm, but it has a broad spectrum which no longer shows dichroism. This result is consistent with the notion that the chromophores get hydrolyzed from the structural protein and removed from the original sites; h) in fresh preparations it appears that retinal is reduced to vitamin A quite rapidly. In such cases a broad, non-dichroic spectrum occurs with maximal absorption of about 330 nm.

Significance to Bio-medical Research and the Program of the Institute: With these studies, understanding of the molecular events leading to visual excitation may be improved. For example, should the preliminary findings be confirmed meta I could be ruled out as a potential candidate among the molecular species that may have decisive roles in the excitation process.

Proposed Course of the Project: Measurements so far performed on rods of the frog retina are to be extended to the cones and then to other vertebrate photoreceptors. It seems particularly worthwhile to repeat Marks' measurements on goldfish cones with the ultimate objective of characterizing primate foveal cone pigments.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/NI-1896
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Photoreceptor System of the Hard-Shell Clam

Previous Serial Number: None

Principal Investigator: Michael L. Wiederhold, Ph.D.

Other Investigators: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.4
Other:	0.1

Project Description:

Objectives: These experiments were undertaken to study a sensory receptor exhibiting primary inhibition.

Methods Employed: Axons in the siphonal nerve of the Hard-Shell Clam, Mercenaria Mercenaria, produce spike responses when the illumination of the inner siphon wall is reduced. Such spike responses from single axons were studied using suction electrodes. The photoresponsive axons in the siphonal nerve may be the axons of photoreceptor cells located in the siphon. Attempts have been made to penetrate the photoreceptors with microelectrodes. This has proven technically difficult, partly because of lack of anatomical knowledge of this system. To alleviate the latter problem, anatomical techniques for injecting fluorescent dye down an axon to its cell body are being developed. This would allow one to trace the photoreceptive axon back to the photoreceptor, identify and locate the receptor and then determine the feasibility of penetrating the receptors with microelectrodes. This technique is being developed using the optic nerve of the horseshoe crab, Limulus.

Major Findings: These photoreceptive axons produce spike responses only when the light is dimmed. In a dark-adapted preparation there is little or no ongoing activity. Although the response is of the "pure off" type, the number of spikes in an off-response and their frequency are strongly related to the previous history of illumination - the brighter the light and the longer it has been on, the greater will be the off-response. If the preparation is kept in total darkness for some time, the off-response to a brief flash is smaller and has a longer latency than that to an identical flash in a light-adapted preparation. The receptive area of a single axon can be localized to a circle of about 85μm diameter on the inner siphon wall, which indicates that each axon innervates only one receptor. To date the technique of electrophoresing dye down axons has not been successful enough to furnish new information on the anatomy of the clam photoreceptors.

Significance to Bio-medical Research and the Program of the Institute: This system is interesting physiologically in that its responses seem to be, in many ways, the "inverse" of those from most photoreceptors described to date, e.g., it responds to the removal of a stimulus and its responsiveness increases with light adaptation. If the axons we have studied are the axons of the receptor rather than second order cells, this preparation offers an opportunity to study the mechanism of primary inhibition in a sensory receptor.

Proposed Course of the Project: The results cannot be interpreted until we know whether these axons are first-order sensory-cell processes or second-order cells. The mechanism of response generation can only be studied directly by penetrating the receptor. Both of these problems require more anatomical information than is available at this time. If the dye technique which is now being developed cannot provide this information, this project will be terminated and some other photoreceptor system will be studied.

Honors and Awards: None

Publications:

Wiederhold, M.L., and Kiang, N.Y.S.: Effects of Electric Stimulation of the Crossed Olivocochlear Bundle on Single Auditory-Nerve Fibers in the Cat. J. Acoust. Soc. Amer. 48: 950-965, 1970.

ANNUAL REPORT

July 1, 1970 through June 30, 1971
Laboratory of Biophysics, Intramural Research
National Institute of Neurological Diseases and Stroke

Robert E Taylor, Acting Chief

University of California Press has corrections in hand for a second printing of "Membranes, Ions and Impulses" as the original printing is nearly exhausted. The Japanese translation is "very popular", the proposed Russian version has encountered publishing difficulties and the plans for a Spanish edition are making only slow progress.

Considerable theoretical work has been done on analysis of voluntary muscular control, excitable ion permeable membranes, mechanical properties of frog muscle sarcolemma, noise in electrolytes and in connection with analogue studies of close packed non-conducting suspensions. The last of these is presumptively related to studies of cortical edema and circulation in the brain and other tissues.

Strength-duration curves, both for cathodal and anodal stimuli and for various temperatures were computed and compared with experiment. Particularly for the case of anodal stimuli the theoretical and experimental results do not agree, which is further evidence that the real axon contains slow processes not included in the equations of Hodgkin and Huxley. These kinds of studies may be a way to investigate these slow processes without the use of voltage clamp techniques.

The theories of threshold fluctuations developed in collaboration with DCRT appear to explain much of the data on this subject in the literature and strongly suggest that threshold (and latency) fluctuations arise mainly from fluctuations in the sodium conductance and not from other putative sources of nerve membrane noise.

Detailed studies were carried out on the effects of both internal and external pH changes on the currents in the squid axon membrane. These results have resulted in the development of several models and significant criticism of some that have been proposed.

The effects of several drugs on squid axon currents were investigated. Among these it was found that neither LSD nor tetrahydrocannabinol (the active principle in marihuana) have any significant effect while Diphenylhydantoin (anticonvulsant) has a significant effect on active sodium currents.

A modification of the dialysis technique for internal perfusion of single fibers for use with single barnacle muscle cells was highly successful. In this technique, a glass tube with a central porous region is passed right through the fiber. Flowing solutions through the tube allows one to change the internal contents of the fiber and to determine influxes of radioactive tracers by collection of effluent. By measuring membrane potential, either with impaled microelectrodes or with internal pipette electrodes, and measuring ion fluxes using ^{22}Na, ^{42}K and ^{36}Cl it was determined that the steep temperature dependence of the resting potential in the barnacle muscle fiber is the consequence of a change in the ratio of the permeabilities to sodium and potassium and is not metabolically dependent.

Another successful method for perfusion of the single barnacle muscle fiber was developed in Chile in which the tendon end of the fiber is left intact. With a spiral platinum wire around the perfusion canulla for supplying current, it was possible to simultaneously voltage clamp, perfuse and make flux measurements with radioactive tracers. These results are still preliminary.

The dialysis technique has also been used with the nerve cord of Myxicola, making radioactive tracer measurements feasible with this preparation. This program has been very productive. In collaboration with the Section on Technical Development and with DCRT the data is recorded on tape with the Micro-LINC 300 and sent via the PDP-12 here to the PDP-10 at DCRT for processing. Using these techniques, successful studies have been carried out on inactivation characteristics of the Na currents, linearity and potassium dependence of instantaneous current voltage curves, sodium substitutes such as Lithium, Ammonium and Guanidinium and the effects of the nerve poison Batrachotoxin.

The program of study of artificial lipid bilayers, particularly when activated with EIM has continued to yield significant results. It had been demonstrated previously in this laboratory that the voltage dependent conductance of the EIM doped lipid bilayer was the result of the opening and closing of individual channels, each of which was ohmic. It has now been demonstrated that the selectivity of the channels to the alkali cations is proportional to the free-solution mobilities. The "closed" channel is not completely closed and it is probably significant that the selectivity of the "closed" channel is the same as the open one.

Several members of the laboratory have contributed chapters to the forthcoming book covering the first Training Course in Biophysics and Physiology of Excitable Membranes given at Woods Hole in 1969. Four members of the laboratory participated in a repeat of this course in 1970. Another laboratory member initiated and assisted in organizing a small conference for extended discussion of membrane ion currents at the University of Alabama in Birmingham.

Serial No. NDS(I)-62 LB/CB 935
1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses.

Previous Serial Number: Same

Principal Investigators: Taylor, R. E,Ph.D., and Latorre, R.,Ph.D.

Other Investigators: Dipolo, R., Ph.D., Rojas, E., Ph.D. and Keynes, R. D., Ph.D.

Cooperating Units: Johns Hopkins University
Laoboratorio de Fisiologia Celular (Chile)
Institute for Animal Physiology, Babraham, England

Man Years

Total:	2.0
Professional:	1.5
Other:	.5

Project Description:

Objectives: Interpretation of the function of excitable mechanisms in terms of ionic permeabilities and the elucidation of the structure and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard methods for measurement of membrane potential with glass microelectrodes or longitudinally inserted pipettes are employed using large single muscle fibers of barnacle. Replacement of internal contents of the cell and subsequent perfusion is accomplished either by a dialysis technique where a glass tube is threaded right through the fiber and has a central porous region or by inserting a glass pipette through an open end of the fiber to the other end with intact tendon and refluxing the solution. In the latter case a platinum spiral is wound around the pipette and serves as a current supplying electrode for voltage clamping. In either case, it is possible to collect the perfusion fluid and measure influx of radioactive tracers, or perfuse with tracer and measure efflux.

Major Findings: We found that the resting potential of the barnacle muscle does not follow the temperature dependence expected from the constant field equation, but varies with increasing temperature by as much as 2 mV/oC. This steep temperature dependence can be explained by assuming that the metabolism contributes a temperature-dependent component to the resting potential (i.e. an electrogenic sodium pump) or that the permeabilities for the different ions (K, Na and Cl) depend on temperature in a differential way and in a direction that would explain our results.

Using inhibitors of the sodium pump we can confirm or discard the first possibility. We tested ouabain, lithium artificial sea water and potassium-free artificial sea water showing that the steep temperature dependence in this cell is not related to the metabolism.

We were able to measure potassium, sodium and chloride fluxes, by means of the dialysis technique. The results obtained show that potassium and chloride permeabilities are very temperature dependent, but sodium permeability is rather independent of temperature.

Scientific Significance: The effect of temperature on the resting potential has been studied in a number of different preparations and has attracted considerable theoretical interest. For cell membranes that are selectively permeable to Na, K and Cl, the resting membrane potential may be described by an equation derived from the constant field theory. Predictions of this equation are only accurate if the permeability constants do not vary with temperature and if active processes do not contribute to the resting membrane potential.

We have demonstrated that in barnacle muscle fibers, in contrast with other preparations, the steep temperature dependence of the resting potential is a consequence only of the variation of the permeability ratio PNa/PK with temperature. In particular, PNa/PK increases when the temperature decreases.

Proposed Course of Project: It has been shown that Ca plays an important role in the muscle contraction. In barnacle muscle, Hagiwara and co-workers were able to demonstrate that the current during an action potential is carried by calcium, rather than sodium, which is the current carrier for the squid axon. A study of calcium fluxes and calcium currents using a combination of voltage clamp and dialysis techniques is anticipated for the following year.

Honors and Awards: None

Publications:

Chandler, W.K. and Meves, H.: Sodium and potassium currents in squid axons perfused with fluoride solutions. J. Physiol. 211: 623-652, 1970.

Chandler, W. K. and Meves, H.: Evidence for two types of sodium conductance in axons perfused with sodium fluoride solution. J. Physiol. 211: 653-678, 1970.

Chandler, W. K. and Meves, H.: Rate constants associated with changes in sodium conductance in axons perfused with sodium fluoride. J. Physiol. 211: 679-705, 1970.

Chandler, W. K. and Meves, H.: Slow changes in membrane permeability and long-lasting action potentials in axons perfused with fluoride solutions. J. Physiol. 211: 707-728, 1970.

Serial No. NDS(I)-62 LB/MA 936
1. Biophysics
2. Mathematical Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations

Previous Serial Number: Same

Principal Investigator: FitzHugh, R., Ph.D.

Other Investigators: Cole, K. S., Ph.D., Lecar, H., Ph.D. Nossal, R., Ph.D., Shaw, J., B.S., M.A. and Rapoport, S. I., Ph.D.

Cooperating Unit: Division of Computer Research and Technology, NIH
Section on Technical Development, NIMH, NIH
Laboratory of Neurophysiology, NIMH, NIH

Man Years

Total:	3.0
Professional:	2.8
Other:	.2

Project Description:

Objectives: To formulate and test theoretical descriptions of (1) excitable ion-permeable membranes, including statistical fluctuations, and (2) voluntary muscular control, using mathematical analysis and computation.

Methods Employed: Mathematical analyses, with particular emphasis on nonlinear differential equations and optimization theory, are being used in conjunction with the available computing facilities.

Major Findings: The computer programs for solving the Hodgkin-Huxley equations and computing thresholds were transferred to the PDP-10 computer and improved in ways:

(1) Better control of roundoff error in the Runge-Kutta integration was obtained by using double-

precision arthimetic for certain variables.

(2) An improved criterion for threshold determination was used. Instead of merely testing whether the peak potential exceeded a preset level, which introduces some arbitrariness when the response is graded, as at higher temperatures, a search was made for the inflection point in the curve of peak potential versus stimulus intensity.

(3) Computed potential curves were monitored on the cathode-ray tube display to eliminate errors during the threshold search procedure.

Strength-duration curves computed for both cathodal and anodal stimuli at different temperatures. The anodal curves agreed in shape with experimental results obtained by Dr. Rita Guttman for stimulus durations up to about 10 msec, but failed to show the further decrease in threshold measured by her for longer durations. This is further evidence that real squid axons contain slow processes missing from the Hodgkin-Huxley equations, and suggests that these experiments provide an alternate way (other than voltage clamp) to investigate these slow processes.

The theory of threshold fluctuations (done in collaboration with Dr. R. Nossal of DCRT) is summarized in two papers which have been submitted for publication. The first paper uses the model of Brownian motion in a conductance-voltage phase plane to describe threshold fluctuations in excitable cells. The theory predicts the distributions of firing fluctuations and latency fluctuations, and relates these statistical phenomena to the continuous electrical noise generated in the cell membrane.

The second paper analyzes the contributions to threshold fluctuations of several putative sources of electrical noise. The different sources of noise lead to different dependences of the threshold fluctuations on experimental parameters such as temperature, ion concentrations, and concentrations of certain drugs. Comparison of the predictions with existing experimental data suggests that threshold fluctuations can best be explained as caused by fluctuations in the sodium conductance of the membrane.

Efforts to solve the optimal control problem for a model of voluntary muscular activity containing a linear series elastic element continue. Iterative computations based on the Pontryagin Maximum Principle failed, apparently because of the extreme nonlinearity of the equations. A simpler direct method is now being tried.

The Hodgkin-Huxley leakage conductance - which they hoped was Cl - is a continuing enigma. It is also of increasing importance because of the possibility that these earliest ions to cross the membrane may be necessary for the subsequent sodium and/or potassium currents. Although this leakage conductance has usually been estimated with an exponential approximation to the capacity sharging transient, there is evidence that the squid axon membrane capacity has a dielectric loss. To take this into account, FitzHugh collaborated with Cole to calculate the transients for such a membrane with the apparent series resistance. Early tests are promising but a routine procedure has not been developed. A preliminary report was given at the Biophysical Society.

Differential equations describing the properties of the stretched sarcolemma of the single frog muscle fiber were developed in collaboration with Stanley I. Rapoport of the Laboratory of Neurophysiology, N.I.M.H., and used by him to estimate elastic moduli from his experimental measurements. The results were reported at the Biophysical Society meeting in February (abstract WPM-L6).

Proposed Course of Project: The study of both membrane stability and membrane fluctuations will be pursued, with the aim of improving present mathematical models of the excitable membranes, which are inadequate in these respects.

Theoretical work on fluctuation phenomena in membranes will be pursued as an adjunct to the experimental studies of the kinetics of membrane conductance. Further study is planned on the origin of membrane 1/f - noise, on the use of noise spectra to distinguish between carrier and channel models of transport in synthetic membranes, and of the effects of membrane noise on spike interval fluctuations in nerve.

The application of dimensional analysis may provide the best way to study the "design" of nerve cells, e.g., the regulation by evolution of quantities like axon diameter, node separation, and nodal membrane area, using a hypothesis of optimal design.

The solution of the equations for optimal muscular control will be sought for a variety of mechanical constraints acting on a single muscle, a pair of antagonists, or more complicated musculoskeletal system. It is hoped that the results may provide at least qualitative predictions for comparison with experiment and the eventual production of a coherent theory of coordinated motion.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 LB/ME 938
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Membrane Dielectric Properties

Previous Serial Number: Same

Principal Investigator: Cole, K. S., Ph.D. and Taylor, R.E,Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	.3
Professional:	.2
Other:	.1

Project Description:

Objectives: The need for more and detailed measurements of membrane dielectric properties remains, particularly in the low frequency range, effect of temperature and drugs and effect of membrane potential.

Methods Employed: The presently available methods for space and voltage clamping the squid giant axon are required as well as the developing techniques for internal replacement and perfusion and the use of accurate bridge networks.

Major Findings: The history and present status of membrane capacity measurements and mostly inadequate analyses and theories were summarized for a conference on physical principles of biological membranes.

Scientific Significance: Detailed information on the impedance of the membrane, particularly the voltage and temperature dependence relations, imposes constraints on any molecular model which is proposed. One example is that the membrane does not have the same dielectric properties as the simple bilipid layers which have been worked on.

Proposed Course of Project: Further experiments are planned and will be done when the possibility presents itself. The techniques are available but the squid are to be had only at Marine Biological Stations. Transportation of squid appears to be feasible but the development of the necessary facilities are beyond the budgetary and manpower resources of the Laboratory. It is hoped that measurements of the effects of membrane potential on membrane capacitance can be carried out in collaboration with Dr. Richard Keynes in England.

Honors and Awards: None

Publications:

Cole, K. S.: Dielectric properties of living membranes. In Snell, F., Wolken, J., Iverson, G.J., Lam, J.,(Eds.) : Physical Principles of Biological Membranes. New York, N.Y., Gordon and Breach, 1970, pp. 1-16.

Serial No. NDS(I)-62 LB/CB 939
1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

Previous Serial Number: Same

Principal Investigators: Gilbert, D. L., Ph.D.,
Ehrenstein, G., Ph.D. and
Taylor, R. E, Ph.D.

Other Investigators: Stillman, I., M.D., Ph.D.,
Fishman, H., Ph.D., Lipicky, R.J., M.D.,
Henkin, R., M.D., Ph.D., Rojas, E., Ph.D.,
Bezanilla, F., Ph.D.

Cooperating Units: Marine Biological Laboratory
Woods Hole, Massachusetts
Laboratorio de Fisiologia Celular
Montemar, Chile

Man Years

Total:	3.5
Professional:	3.0
Other:	.5

Project Description:

Objectives: To study the ionic current flow across the nerve membrane without the complications of excitation and propagation in terms of individual ion currents and to observe the effects of changes in normal internal and external ionic concentrations. The long range objectives are the interpretation of nerve function in terms of ion movements and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed with the addition of control of the internal environment by replacement and perfusion.

Major Findings: The squid nerve under voltage clamp conditions provides an excellent preparation for determining the action of psychotomimetics. Synthetic tetrahydrocannabinol (THC), an active ingredient of marihuana (Holister, Science 172: 21, 1971) and lysergic acid diethylamide (LSD) were chosen as the psychotomimetics in our study. No effect of either THC or LSD was noticed on the electrical properties of the voltage-clamp squid giant axon.

The effect of external pH upon the voltage clamped currents of the squid giant axon was investigated. Current-voltage plots were obtained directly by using the ramp voltage clamp technique (Fishman, Biophys. J. 10: 799, 1970). Ramp slopes of 68 mv/msec and 0.56 mv/msec were used to measure the fast sodium and slow potassium currents, respectively. The sodium currents decreased as the pH was decreased. A plot of the percentage decrease in the maximum sodium current versus pH, yields a titration curve with pK of about 6.5. The rate at which the maximum sodium currents decreased was pH dependent. The sodium currents decreased more rapidly at lower pH values than at higher ones. At a pH of 6, the current began to decrease after two minutes and leveled off within ten minutes. A small voltage shift in the maximum sodium current as a function of pH was observed. A computer analysis of the Hodgkin-Huxley model of the squid giant axon under our ramp clamp conditions suggest that this small voltage shift is due to two effects: The first effect is due to the binding of hydrogen ions on the negative sites of the external surface of the nerve membrane; The later second effect is due to the change in the time constants of the channels responsible for the sodium entry. The magnitude of the potassium currents are not greatly affected until the pH decreased below a value of five.

The main effect of decreasing internal pH in a squid axon is a decrease in the amplitude of the transient sodium current. The steepness of the curve of relative transient conductance versus pH and the observed increase in the time-to-peak of the transient sodium current can be explained by a model in which several protons can bind to a single transient channel, causing successive decreases in the channel conductance and in the rate constant for channel equilibrium.

Diphenylhydantoin (DPH) possesses both anticonvulsant and antiarrhythmic properties. The possiblility that both effects may share a common mechanism led to this study of the effect of DPH upon the voltage-dependent currents of the squid giant axon. The voltage clamp technique was used with both the step and ramp clamp methods at about 5°C. Doses of 10 μM and 50 μM reduced the inward sodium current by about 50 percent. When the dose was reduced to 1 μM, this inhibition was no longer noticeable. Use of the ramp clamp method showed that at the high dose of 50 μM, the reduction of the sodium current began within three to five minutes after initial exposure and produced its full effect after 30 minutes. DPH does not change the resting membrane potential and only slightly decreases the potassium current. Reduction in the sodium current may explain some of the actions of DPH.

Scientific Significance: Our studies with the psychotomimetics, THC and LSD, indicate that their effects are not due to any action directly on the mechanisms responsible for nerve propagation. Since these drugs have such effect, our negative findings are important ones.

It appears from our pH studies that there is a controlling site on the nerve membrane which when bound with hydrogen ions blocks the sodium channel. This site has an apparent pK of about 6.5. This effect is much more important than any neutralization of the external negatively charged membrane surface by hydrogen ions at low values of pH.

Our experiments indicate that DPH interferes with the fast sodium channel. This result might explain in part the anticonvulsant action of DPH. One of the most important properties of a conducting channel in a membrane is its charge distribution. This property is crucially related to channel selectivity, and is also of interest in regard to channel gating mechanisms and membrane stability. The finding that each channel has several sites which can bind protons is a strong condition that must now be imposed on channel models.

The effect of internal pH on the transient sodium conductance also provides a critical test of a model recently proposed by W. G. S. Stephens (Nature 224: 547. 1969). In order to explain the increase in transient sodium conductance caused by depolarization, Stephens proposed that depolarization increases the outward flow of hydrogen ions, and that the increased transient sodium conductance occurs because of the displacement of blocking calcium ions by the hydrogen ions. According to this model, if the outward flow of hydrogen ions is increased by increasing the internal hydrogen ion concentration, the transient sodium conductance should also increase. We have found that under this condition, the transient sodium conductance decreases. This observation provides a clear refutation of Stephens' model.

Porposed Course of Project: It is planned to study further the effect of pharmacological agents and trace metals upon the voltage clamped currents of the squid giant axon.

Honors and Awards:

Daniel L. Gilbert was elected to be a member of the Advisory Committee to Membrane Biophysics Group, Biophysical Society for 1971.

Daniel L. Gilbert was appointed to the Nominations Committee, District of Columbia Sigma Xi Chapter for 1971.

Daniel L. Gilbert was asked to write a leading review article on oxygen for the journal, Anesthesiology.

Daniel L. Gilbert and Gerald Ehrenstein were asked to write a review paper on surface charges for the annual series, Progress in Biophysics and Molecular Biology. This request was turned down because this topic is being covered in Daniel L. Gilbert's chapter on "Fixed Surface Charges" which will appear in W. J. Adelman's book entitled, Biophysics and Physiology of Excitable Membranes.

Publications:

Cole, K. S. and Gilbert, D. L.: Jet propulsion of squid. Biol. Bull. 138: 245, 1970.

Gilbert, D. L. and Ehrenstein, G.: Use of a fixed charge model to determine the pK of the negative sites on the external membrane surface. J. Gen. Physiol. 55: 882, 1970.

Stillman, I. M., Gilbert, D. L., and Robbins, M.: Monactin does not influence potassium permeability in the squid axonal membrane. Biochim. Biophys. Acta 203: 338, 1970.

Gilbert, D. L. and McCutcheon, F. H.: Respiratory media (Table 7) and Respiratory molecules (Table 8). In Altman, P. L. and Dittmer, D. S. (Eds.): Respiration and Circulation. Bethesda, Md., Federation of the American Society for Experimental Biology, 1971, p. 13.

Ehrenstein, G. and Fishman, H. M.: Experimental evidence against hydrogen-calcium competition model for activation of electrically excitable membranes. Nature (in press).

Fishman, H. M.: Direct and rapid description of the individual ionic currents of squid axon membrane by ramp potential control. Biophys. J. 10: 799-817, 1970.

Bezanilla, F., Rojas, E. and Taylor, Robert E: Sodium and potassium conductance change during a membrane action potential. J. Physiol. 211: 729-751, 1970.

Serial No. NDS(I)-62 LB/I 940
1. Biophysics
2. Instrumentation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ionic Permeabilities of Excitable Membranes: Effects of Chemical Agents

Previous Serial Number: Same

Principal Investigators: Binstock, L., B.S., M.S.,
Lecar, H., Ph.D.

Other Investigators: Goldman, L., Ph.D., Stillman, I., M.D.,Ph.D.
Latorre, R., Ph.D.

Cooperating Unit: University of Maryland Medical School
Division of Computer Research and Technology, NIH
Section on Technical Development, NIMH,NIH

Man Years

Total:	3.1
Professional:	1.8
Other:	1.3

Project Description:

Objectives: The voltage clamp technique allows measurements of ionic current flows across nerve membrane as a function of voltage and time. These parameters have been combined into a set of empirical equations capable of expressing nerve membrane activity, but the physical meaning of the equations is unknown. It is hoped that the use of various chemicals known to effect nervous activity may contribute to understanding the physical mechanisms of the membrane. Irrespective of the chemical properties of the substances, this may permit dissection of the lumped parameters of the equations as well as a correlation of known chemical properties with the observed effects.

Methods Employed: Previously described voltage clamping techniques are used, either with or without perfusion, with the giant axon of the marine worm Myxicola infundibulum. For perfusion, a cannula is first inserted into the axon and passed right through to the other side of the chamber, removing most of the axoplasm. It is then used as a guide to insert the "piggy back" electrode, which is a combination current supplying and voltage measuring device, and finally pulled back to serve as the inlet cannula.

A new chamber has been used to improve the internal perfusion technique. The technique is similar to that used for barnacle. The axon is placed over a moveable cannulae, one on each end. The perfusion canula then is inserted from one end through the larger cannula and the double ("piggy-back") electrode is inserted from the other end. Dialysis of the Myxicola giant axon has been successfully worked out for tracer studies.

Data is obtained with the Micro-LINC 300 for both on and off line analysis of the voltage clamped axons. Data sampling time is routinely 40 or 10 microseconds with a 1.0 microsecond capability. Data from LINC tape is sent via the PDP-12 to the PDP-10 at DCRT for additional processing. Some changes in the program for sending data over to the PDP-10 have been made, making it much easier.

Major Findings: Inactivation characteristics of the Na current were studied under voltage clamp in Myxicola giant axons by observing the effects of pre pulses of varying amplitude (V) and duration (t) on the Na current produced by a test pulse of constant amplitude. All measurements were made using compensated feedback. The time constant tau $_h$ as a function of voltage shows behavior similar to that reported for squid and muscle. The mean value of h at the natural resting potential is about 0.9. Inactivation in Myxicola can therefore, be described by the methods of Hodgkin and Huxley.

Continued studies of sodium substitutes, Li and NH_4, in the Myxicola giant axon, show behavior similar to that found in squid. The permeability of NH_4 to Na is somewhat less than in squid. It is a depolarizer, depolarizing the resting potential 20 - 25 millivolts similar to that in squid. The potassium currents are decreased. The post anodal action potential is about half of what it is in squid. The kinetics - - time constants - - are the same as for sodium indicating that NH_4 moves through the transient or fast channel. Leakage seems to be somewhat less than with Na. Li depolarizes the axon by only a few millivolts. The kinetics are the same as that for sodium. Tetrodotoxin (TTX) blocks Li currents. Leakage seems to be less than with Na. No work as yet has been done on NH_4 as a potassium substitute. (NH_4 goes through either the early (transient) channel or the later (steady state) channel in squid).

Preliminary studies have been done using Batrachotoxin (BTX) on the Myxicola giant axon with the results somewhat similar to that obtained in the squid axon. 10^{-6}M BTX artificial sea water was used which irreversibly depolarized the axon about 11 millivolts. This was returned to normal with the application of TTX. Assuming a standard dosage of unity, the dosage used in these experiments was about 0.75.

It appears that some additional transient channels were produced by the BTX or that the transient channels were permanently held open. The results seem to indicate that the BTX increases the resting sodium conductance of the Myxicola axon membrane.

The prevailing view of the mechanism of nerve excitation at present is that of functionally distinct sodium and potassium pathways. Each pathway is characterized by its own ion transport process which can be described independently of the state of the various voltage-dependent membrane activation processes. Thus a rapid measurement of ionic currents, during a time interval too short for the membrane conductances to change, will yield the driving force relation of the ion transport process.

The driving-force relation is given by the instantaneous current-voltage curves of the fully activated sodium and potassium channels. Such measurements are difficult to perform because of the very short times required for the fully activated system to relax to the nonconducting state. Using an improved rapid voltage-clamp technique, we are measuring instantaneous current-voltage relations on Myxicola giant axons.

The experiments are designed to answer the following questions:

(1) Is the instantaneous current-voltage relation linear, as reported for the squid giant axon, or does it exhibit rectification reported for single nodes of Ranvier?

(2) How does the form of the driving-force vary with the concentration of the dominant ion?

(3) Is there evidence for current saturation? Such saturation would indicate the rate-limiting step of the transport process.

Preliminary results show the relations to be nonlinear, and the dependence on concentration to be consistent with a steady-state electrodiffusion process. Instantaneous currents have been measured over a range of potentials equal to 300 millivolts on either side of equilibrium with no consistent demonstration of current saturation.

Scientific Significance: A thorough understanding of the relationships between ionic concentration's inside and outside of the axons and of the voltage-dependent ionic conductances are necessary to understand the normal functioning of excitable membranes. The various studies on the *Myxicola* giant axon are making it possible to get some kind of insight into the mechanism of the excitable membrane.

Proposed Course of Action: Dialysis and internal perfusion will be continued with voltage clamping. Various tracers will be used.

The study of "sodium-substitutes" and "potassium substitutes" of the quaternary-ammonium ion type and others will be continued to see what their effect is on the membrane. To increase the understanding of the effects of heavy water, experiments are planned with variations in D_2O concentrations, ionic strength, temperature and pH. These are all to be done on the *Myxicola* giant axon. BTX will continue to be studied at standard dosages and in greater detail. The use of other drugs similar to TTX usch as DDT, and tannic acid as well as anesthetics are also planned.

The PDP-10 will continue to be used in greater detail to make analysis of the various drugs studied almost "on-line".

Honors and Awards: None

Publications:

Binstock, L. and Goldman, L.: Instantaneous potassium current-voltage relations in *Myxicola* giant axons. *J. Physiology* (London) 1971 In Press.

Serial No. NDS(I)-65 LB/ME 1240
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Molecular Biophysics - Physical Properties of Membranes and Simple Membrane-like Systems

Previous Serial Number: Same

Principal Investigators: Ehrenstein, G.,Ph.D., Lecar, H.,Ph.D., Stillman, I.,M.D.,Ph.D., Latorre, R., Ph.D.

Other Investigators: None

Cooperating Units: Division of Computer Research and Technology, NIH

Man Years

Total:	2.3
Professional:	1.7
Other:	.6

Project Description:

Objectives: To study physical properties and molecular structure of synthetic systems related to natural membranes. To examine related molecular properties of membranes with newer physical techniques.

Methods Employed: Synthetic bilayer membranes are prepared from beef brain extracts, oxidized cholesterol, or other lipids between two salt solutions. The steady state and fluctuating electrical properties of the membranes are measured before and after the application of an activating factor, such as EIM (probably composed of protein and other material). These measurements employ calomel electrodes, low-noise amplifiers with high input impedances, and suitable averaging and recording devices.

Magnetic resonance spectroscopy is used to determine structure of systems relating to membranes, such as the hydrocarbon moiety of isolated biological membranes or proteins.

Major Findings: When a moderate amount of EIM is added to a lipid bilayer, the conductance increases by several orders of magnitude and the current-voltage curve has a negative resistance region that is quite similar to that found for excitable cells.

We have previously investigated the properties of this negative resistance by observing the electrical behavior of membranes containing very small amounts of EIM. We found that the conductance is limited to a few discrete levels and changes abruptly from one level to another. From our study of these fluctuations, we concluded that the EIM-doped bilayer contains ion-conducting channels capable of undergoing transitions between two states of different conductance.

This EIM system offers a unique opportunity to determine ionic selectivity directly from the relative magnitude of the current steps for different ions at the same membrane potential. We have made such measurements for a number of univalent cations. For the alkali cations, in particular, the selectivities were also measured by the conventional biionic potential method. Both methods indicate that the alkali cation selectivities are proportional to the respective free-solution mobilities.

Nuclear magnetic resonance studies of lyophilized myelin have indicated considerable freedom of motion within the hydrocarbon moiety. Furthermore, sharp thermal transitions were observed, suggesting that lyophilized myelin is in a liquid crystalline state.

Scientific Significance: The two salient features to be explained in order to provide a molecular understanding of excitability in natural membranes are:

1. potential-dependent ionic conductance and
2. ionic selectivity

The EIM-activated bilayer is a synthetic excitable membrane with the useful attribute that properties of individual channels can be experimentally determined. We have shown that in this system, the potential-dependent ionic conductance arises from the opening and closing of the ionic channels. We have now also shown that cationic selectivity in the EIM system is proportional to free-solution mobility.

Thus, EIM is rather similar to squid axon as regards property 1 above, but quite different as regards property 2. This demonstrates that the two properties are not inherently coupled, and that there may be separate mechanisms for them in natural excitable membranes.

Proposed Course of Project: The EIM system can be used to make other comparisons between properties of individual channels and properties of macroscopic membranes. We plan to persue this approach particularly in the determination of the kinetics of EIM channels. These measurements should also indicate whether the channels are independent.

We also plan to determine the effect on the EIM system of changes in temperature and pH. One result should be a better determination of barrier height, which is useful in describing kinetic properties.

Honors and Awards: None

Publications:

Ehrenstein, G.: Excitability in lipid bilayer membranes. In Adelman, W.J. (Ed.): Biophysics and Physiology of Excitable Membranes. New York, N.Y., Van Nostrand Reinhold Co. (in press).

Lecar, H., Ehrenstein, G., and Stillman, I.: Detection of molecular motion in lyophilized myelin by nuclear magnetic resonance. Biophysical J. 11: 140-145, 1971.

Annual Report

July 1, 1970 through June 30, 1971

Laboratory of Experimental Neurology, Intramural Research
National Institute of Neurological Diseases and Stroke

William F. Caveness, M.D., Chief

The official establishment of the Laboratory within the Intramural Program, NINDS, NIH, was completed on April 4, 1969. The renovations in the assigned space in Building 36 were completed in March 1970. The activities for the past 15 months represent the continuation of the investigations begun some ten years ago within the Department of Neurology, Columbia University, College of Physicians and Surgeons.

The current scientific effort may be described within three areas:

1) Radiation Biology: Since 1961 the Columbia Laboratory, in conjunction with the Brookhaven National Laboratories, has studied the effect of x-irradiation on the cerebral cortex of the young adult macaca mulatta. A single exposure of 3,500 rads has been employed. The exposure site was initially the right hand-face area, but since 1966 it has been the right visual cortex. The former provided dramatic clinical findings in focal seizures and hemiparesis but the latter provides a more precise measurement of functional changes as expressed in the photic evoked response. Attention has been directed to the ultrastructure evidence for cellular changes in carbohydrate and protein metabolism within days of exposure, alteration in the dendritic plexus within 4 to 12 weeks, and radionecrosis in the exposed area within 4 to 5 months following irradiation. In addition, we have observed the functional and structural changes in the irradiated and non-irradiated cortex over prolonged periods, up to 36 months, following exposure. Long term functional alterations have included bilateral depression of evoked response and behavioral blindness. The long term structural changes have included periodic brain swelling, that in some instances has been incapacitating, and retrograde transsynaptic degeneration within the visual system. The investigation of these long term effects is continuing with additional monitoring of intracranial pressure.

A fresh hypothesis, that irradiation so alters nervous and supporting elements that existing viruses may replicate and eventually contribute to the destruction of the exposed tissue, was tested. The results were negative for CNS foamy virus and other simian viruses as determined by serologic and tissue culture studies. A further test of this hypothesis will include a search for slow and latent viruses with appropriate techniques.

The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and space exploration.

2) Ontogeny of Focal Seizures in the Macaca Mulatta: A significant feature of this investigation has been the apparent dependency of the immature motor cortex on thalamic structures in the development and propagation of the paroxysmal discharge. During the past year this concept has been tested by a) the interruption of the cortico-cortical circuitry surrounding the activated right hand-face area, and b) the interruption of the cortico-thalamic circuitry beneath the activated area in the newborn monkey. The results were compared with the findings after similar procedures in 24 month old monkeys. Both supported the working hypothesis. The anatomical substrate for the seizure activity in the newborn cortex is being investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. These studies will be extended to provide a definitive comparison with the cortical development at 24 months of age.

3) Regional Blood Flow Studies: A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. In lieu of duplicating their efforts, it seemed wise to determine the natural course of events by the use of regional blood flow techniques. For the latter we are using the ^{14}C-labeled antipyrine method at sequential intervals after the induction of the seizure. A preliminary survey in 12 monkeys has indicated the appropriateness of this method. There are two advantages in exploring subcortical mechanisms in the newborn. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated then one may more appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe. As in the other procedures, the findings in the newborn will be compared with those at 24 months of age.

From such studies there should be improved understanding and management of focal seizures in children and adolescents.

The staff of this Laboratory is limited to five permanent positions and one or two temporary appointments. For the effective conduct of its investigations it is dependent upon the fruitful collaboration of colleagues in allied or disparate disciplines. Of especial importance are co-workers at the Brookhaven National Laboratories, the Neuroanatomical Laboratories at Harvard, Johns Hopkins University, the Section on Infectious Diseases, Perinatal Research Branch, NINDS, and the Laboratory of Cerebral Metabolism, NIMH.

For essential supplies and procedures the Laboratory is dependent upon contracts with Bionetics Research Laboratories regarding newborn monkeys and Harvard University regarding processing and assistance in interpreting anatomical specimen.

Serial No. NDS(I)-69 LEN/OC 1693
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Functional and Structural Alterations Following X-irradiation of the Cerebral Cortex in the Monkey

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators:
Motohiro Kato, M.D.
Kenneth H. Hess
Arland L. Carsten, Ph.D.
Perry Black, M.D.
John L. Sever, Ph.D., M.D.
Luiz Horta-Barbosa, D.V.M.

Cooperating Units:
Brookhaven National Laboratories
Johns Hopkins University School of Medicine
Section on Infectious Diseases, PRB, NINDS

Man Years

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description

Objectives: To determine the pathogenesis of early and late effects of irradiation on the central nervous system.

Methods Employed: The right visual cortex, in the young adult macaca mulatta, is exposed to 3,500 rads of x-irradiation in a single dose. Functional changes are monitored by evoked electroencephalographic responses from photic stimulation of the retina prior to and at weekly intervals following irradiation. The stimuli are programmed to provide single light flashes that may be averaged and a series of frequencies that range from three to thirty flashes per second in ten second epochs. The evoked response from the irradiated and non-irradiated visual cortex is led through a two-channel Grey-Walter, low frequency analyzer that provides histograms for the abundance of activity, or amplitude, for ten seconds at each of the frequencies employed. Thus one can compare directly the amplitude of the evoked response from the irradiated cortex with that from the homologous non-irradiated cortex. The averaged evoked response of a series of thirty-two flashes at one second intervals has provided a more sensitive indicator that is especially useful when the differences between the irradiated and non-irradiated side are less pronounced.

Sequential anatomical examinations have been carried out by sacrificing three animals at 72 hours, 1, 4, 12, 20, 28, 44, and 144 weeks for routine, Golgi-Cox and electron microscopic preparations. Six animals have served as controls.

Major Findings: In the first ten months the functional changes included an initial and secondary unilateral depression in the photic evoked response in the right visual cortex. There was ultrastructure evidence for changes in carbohydrate and protein metabolism within three days of exposure, alteration in the dendritic plexus within 4 to 12 weeks, and radionecrosis, in the exposed area, within 4 to 5 months following irradiation. (Special Issue of Brain Res. 7: 1-120, 1968) In nine additional preparations, long term functional alterations, up to 36 months, have included bilateral depression of evoked response and behavioral blindness. The long term structural changes have included periodic brain swelling, and retrograde transsynaptic degeneration within the visual system.

Split brain preparations will afford additional insight into the mode of transmission from the irradiated to non-irradiated cortex. For the latter there is a current series of 18 animals under observation including 3 non-irradiated controls, 6 irradiated controls, 6 with corpus callosum and optic chiasm section followed by irradiation and 3 with sections but no irradiation. Of primary interest will be the appearance or lack of appearance of the bilateral depression in the evoked response amplitude and the extent of degenerative changes in the visual pathways at and beyond the ninetieth week of observation.

An initial test of the concept that a latent virus may be present and, activated by the radiation, contribute to the developing pathology in the exposed brain. Serologic and evoked response studies were conducted at 4 week intervals. At 4 weeks, 12 weeks and 22 weeks, that represent known stages in the developing pathology, respectively, two animals received biopsies from the irradiated and homologous non-irradiated brain for tissue culture and electron microscopy, prior to sacrifice and routine histopathology. The two control animals received biopsies and sacrifice, at 22 weeks.

Within this period of observation, one of the irradiated animals, at 22 weeks, revealed a foamy virus in tissue culture. The remainder were negative for foamy and other simian viruses.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and space exploration.

Proposed Course: A logical extension will be a more detailed search for primary and secondary changes based upon the observations to date. Specifically, further attention will be directed to ultrastructure and enzyme changes in the period immediately following exposure; evidence of reparative processes prior to radionecrosis at 4 to 5 months; the occurrence of recurrent edema, by monitoring the intracranial pressure; and the remote effects as a result of transsynaptic, retrograde degeneration.

The course of structural changes, proceding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). These possibilities should be energetically explored with new series of irradiated and control monkeys.

Honors and Awards: None

Publications:

Carsten, A. L., Caveness, W. F., Roizin, L., and Machek, J.: Bilateral depression in photo-evoked response as a late effect of unilateral visual cortex x-irradiation. Brain Res. 20: 389-400, 1970.

Serial No. NDS(I)-69 LEN/OC 1694
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ontogeny of Focal Seizures

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators:
Motohiro Kato, M.D.
Kenneth H. Hess
Raymond D. Adams, M.D.
Paul I. Yakovlev, M.D.
Thomas L. Kemper, M.D.

Cooperating Units: Harvard University Medical School

Man Years

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

Objectives: To better understand the evolving seizure pattern in the developing brain.

Methods Employed: Focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution into the hand-face area of the motor cortex of the macaca mulatta at successive age levels.

The onset and elaboration of the attack pattern is simultaneously recorded by electrocorticography, electromyography, and clinical observation.

The cerebra of the experimental, and a control series of animals, are examined for the level of development in cells, neuropil and myelin with attention to the motor cortex and subcortical tracts that are deemed relevant.

Major Findings: A significant feature of this investigation has been the apparent dependency of the immature cortex on thalamic structures in the development of the paroxysmal discharge. This concept is currently being tested by the interruption of the cortico-thalamic circuitry. Begun in the newborn, this procedure will be carried out at successive age levels to demonstrate not only the dependence of the immature cortex on subcortical structures but also the evolving lateral association pathways within the developing cortex.

Traditionally we think of the cortex as of primary importance in propagation of focal paroxysmal activity. This may not be the case in the newborn in which the intracortical lateral association pathways are not yet adequate for this function. Rather, the locally activated cortex, e.g., the face area in the precentral gyrus, may invoke paroxysmal activity in specific and non-specific nuclei of the thalamus, and, or other subcortical structures in which the propagation then takes place along the subcortical representations of face, hand, arm, and leg, with a reflection back to the corresponding cortical areas and, via the pyramidal and extrapyramidal systems, a clinical expression of the evolving seizure.

To test this concept, the cortical-subcortical pathways beneath the face area in the precentral gyrus were sectioned in eight newborn monkeys. The undercut cortex was then injected with 25,000 units of crystalline penicillin in 0.025 cc aqueous solution. The paroxysmal activity recorded from an array of cortical electrodes developed within the undercut area and, except for limited and transient expression, did not spread beyond this region, suggesting a marked dependency upon the subcortical circuitry for propagation of the focal abnormality. Another procedure, in eight newborn animals, involved a subpial section of the cortex on four sides that separated the face area from the remainder of the precentral gyrus and the frontal cortex anterior to the arcuate sulcus. When penicillin was injected into the circumsected cortex the paroxysmal activity developed and spread beyond the cortical sections in the superior and inferior motor strip and to other regions of the cortex with concomitant clinical expression of the seizure, suggesting a limited dependence upon the continuity of the cortex for propagation of the abnormal activity.

Both procedures were carried out in monkeys at 24 months of age. The results to date, in contrast to the findings in the newborn, suggest a far greater dependence on the cortex for the development and propagation of the paroxysmal activity.

Significance to Bio-Medical Research and the Program of the Institute: The results from this investigation should improve the understanding and management of the age dependent seizure patterns in humans.

Proposed Course: Continuation of the study of the relative significance of cortex and subcortical structures at the successive age levels in the development and propagation of the seizure discharge. See new project number 1897.

Honors and Awards: None

Publications:

Caveness, W. F., Echlin, F. A., Kemper, T. L., and Yakovlev, P. I.: The propagation of focal paroxysmal activity in the newborn macaca mulatta. Trans. Amer. Neurol. Ass. 95: 91-96, 1970.

Serial No. NDS(I)-69 LEN/OC 1695
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cortical and Subcortical Recording of the Electrical Reflection of Sleep

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: A search for the origin of the principal forms that are reflected in the electroencephalogram during various phases of sleep.

Methods Employed: The "generators" of the electrical forms of sleep have been sought through surface and depth recording in the monkey. This has begun with observations in the young macaca mulatta, in which the forms are most prominent, prior to the full development and integration of the cortex.

The cortical recording is obtained from frontal, temporal, and occipital electrodes, bilaterally. The depth recording is from midline thalamic structures, brain stem reticular formation, and the hippocampus. The observations are carried out during two hours of the normal sleeping period, daily for seven days after the electrode implantation.

Major Findings: The preliminary findings implicate the reticular formation and midline thalamic structures with the deeper phases of sleep and the hippocampus with drowsiness. The limited availability of time and other demands have prevented the anticipated extension of this project during the past year.

Significance to Bio-Medical Research and the Program of the Institute: The results should add to the understanding of the physiological parameters of normal sleep and long range, to the understanding of the precipitation factors in disease processes, e.g., convulsive seizure coincident with sleep.

Proposed Course: Refinement of techniques and extension of observations to puberty.

Honors and Awards: None

Publications:

None

Serial No. NDS(I)-71 LEN/OC 1897
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Regional Blood Flow Studies

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators:
Motohiro Kato, M.D.
Thomas L. Kemper, M.D.
Louis Sokoloff, M.D.

Cooperating Units:
Harvard University Medical School
Laboratory of Cerebral Metabolism, NIMH

Man Years

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. In lieu of duplicating their efforts, which are limited by the position of the probes, it seemed wise to determine the natural course of events by the use of regional blood flow techniques, using the ^{14}C-labeled antipyrine method developed by Sokoloff et al, at sequential intervals after the induction of the seizure. A preliminary survey has indicated the appropriateness of this method. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe.

Honors and Awards: None

Publications:

None

Serial No. NDS(I)-71 LEN/OC 1898
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Activation of Unilateral Visual Pathways

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators:
Louis Sokoloff, M.D.
Charles Kennedy, M.D.
Motohiro Kato, M.D.
Kenneth H. Hess
Thomas L. Kemper, M.D.

Cooperating Units:
Laboratory of Cerebral Metabolism, NIMH
Harvard University Medical School

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description

Objectives: To delineate the alteration in blood flow within the various components of the visual system after photic stimulation.

Methods Employed: This study will utilize three split brain preparations, young adult macaca mulatta with complete section of corpus callosum and optic chiasm, that have been observed as to their photic evoked response over a period of 12 months. The monthly routine has included unilateral, and bilateral retinal stimulation. These and three control animals will be subjected to ^{14}C antipyrine regional blood flow studies following stimulation of the right eye for three minutes. Three additional control animals will receive ^{14}C antipyrine studies without visual stimulation. Note: The split brain preparations will afford an unique opportunity to compare the two hemispheres after unilateral stimulation.

Major Findings: None to date.

Significance to Bio-Medical Research and the Program of the Institute: Fresh insight into the relation of neurological function to alteration in blood flow, as regards the visual system. Exploration of a projection to the contralateral hemisphere through brain stem mechanisms.

Proposed Course: Initiate and complete the plan described above.

Honors and Awards: None

Publications:

None

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Stroke

Donald B. Tower, Chief

The program of the Laboratory comprises currently some twelve projects that continue to be productive and to show significant advances. Such progress reflects the highly successful catalysis achieved between imaginative conceptions of projects by the five senior staff and skillful, dedicated prosecution by the twenty or more research assistant and associate personnel of the Laboratory. The personnel roster remains full, with 8 research associates and fellows (including 3 NINDS Special Fellows and 1 NIH International Fellow) and 4 guest workers and visiting scientists (including 2 predoctoral graduate students), and has been augmented from other NIH intramural programs by 5 investigators devoting substantial proportions (usually over 50%) of their time to projects in this Laboratory. Although most of these young men and women come from domestic institutions, an international flavor is provided by those from Germany, Argentina, Hungary and Taiwan (and many more are turned away for lack of space and slots). The ability of the Laboratory to attract capable and productive young investigators is the hallmark of a successful program, but the success is in turn a consequence of the stimulating and fruitful collaboration of these young scientists. Unquestionably this collaborative catalysis is a most essential ingredient in the formula for a good program and for an enviable record of "graduates."

Clearly no active research endeavor flourishes in isolation. The Laboratory continues to engage in extensive collaboration with other NIH and outside groups. During the past year in response to requests, the Laboratory rendered substantial assistance to the National Air Pollution Control Administration laboratory in Cincinnati and to the National Institute of Environmental Health Sciences laboratory in North Carolina in connection with studies on the effects of ozone and of methyl mercury, respectively, on the mammalian nervous system. Services nationally and internationally continue to be provided by the Laboratory's Section on Lipid Chemistry for diagnostic tests, identification of carriers, and genetic counselling in the various sphingolipid storage diseases. The Laboratory has also benefitted from the contributions to its own projects by the Weizmann Institute of Science (Rehovoth, Israel), the Scripps Institute of Oceanography (La Jolla, Calif.), the Karlsen Shipping Co. Ltd and the Canadian Fisheries Research Board (New Harbour, Nova Scotia), the Mote Marine Laboratory (Sarasota, Fla.), the Department of Neurology at the University of Pennsylvania (Philadelphia), the Department of Neurosurgery at the Roswell Park Memorial Institute (Buffalo), the Biomedical Engineering Branch of DRS-NIH, laboratories within NCI, NEI, NHLI and NIMH, and within NINDS, the Surgical Neurology Branch and the Laboratory of Neuropathology and Neuroanatomical Sciences. The list is not exhaustive and can barely acknowledge the value of the various collaborative contributions to Laboratory projects.

Individual recognitions for Laboratory staff members provide another index of accomplishments. During the past year three senior staff members continued to serve on the editorial boards of Experimental Neurology, of Biochimica et Biophysica Acta, and of the Journal of Neurochemistry (in this last case as chief editor). The Laboratory was honored to have one of its senior staff selected to give the 1970 annual G. Burroughs Mider Lecture at NIH, and another chosen to present the William G. Lennox Guest Lecture at the 1970 annual meeting of the American Epilepsy Society. The research accomplishments of one senior staff member have been featured in the ACS Chemical Innovators series and two network TV shows. Another senior staff member selected by the National Academy of Sciences for a 3-month exchange program with the Yugoslavian Academy of Sciences is conducting research in the Brain Research Institute at the Adriatic marine station at Kotor. Again the list is not exhaustive but is indicative of the stature achieved by Laboratory staff as a consequence of their contributions to neurochemistry.

The individual project reports which follow summarize the current progress in these contributions. The variety and growing diversity are healthy signs of probings at the frontiers of knowledge, as exciting new leads develop. Despite the superficial diversity, the basic emphasis of all Laboratory programs continues to be on studies of neural membranes. The brief comments included here indicate some of the facets that are receiving current attention.

Certain projects continue to exploit comparative and/or ontogenetic approaches, as exemplified by analytical and enzyme studies on samples of whale brain, on responses of elasmobranchs and teleosts to substituted quinoline anesthetics, on wax ester metabolism in marine crustaceans, on plasma membrane enzymes in various species, on glycoproteins and myelin basic proteins during maturation of the nervous system, and on glycolipid metabolism in various cell lines in tissue culture. All such studies are designed to identify principles of structural and functional organization that often are obvious only from interspecies comparisons and from careful developmental studies. One of the most intriguing has been the observation that the turnover of myelin basic protein (labeled in the single tryptophan residue) is unexpectedly high in the CNS of mature rats. In the face of such data, the long-standing concept of myelin as a stable structural element is no longer tenable. Moreover these and related studies on details of factors involved in myelination give promise of more successful attacks on the problems of demyelination in multiple sclerosis and related disorders. Another very promising set of studies involves the developmental aspects of glycoprotein metabolism. The Laboratory has had a lengthy experience with the glycolipids and now is in the forefront of laboratories embarking on a somewhat parallel investigations of glycoproteins. Together these two groups of compounds subserve many vital receptor and transport functions at cell surfaces, with specificity often conferred by the nature(s) of the carbohydrate moieties. The glycoproteins pose a particularly difficult problem because of their extreme microheterogeneity; hence, the resort to comparative and ontogenetic approaches for additional insight.

A number of the Laboratory projects continue to carry a major emphasis on enzymatic mechanisms. The most extensive of these projects comprises the studies on Na-K-ATPases, now at a stage which is providing numerous important kinetic data on catalytic sites for the protein phosphokinase and phosphatase activities. The emphasis in these studies is now shifting to structural aspects, notably the amino acid composition of the phosphorylated peptide. Progress is also being made in purification of several of the catabolic enzymes defective in the sphingolipid storage diseases, with the eventual dual goals of identifying the exact molecular nature of the derangements and of utilizing the pure enzymes in experimental therapy. A very comprehensive survey of the properties of enzymes characteristic of plasma membranes has been completed and an evaluation of species differences is under way. These studies should prove particularly useful for the next stage in the project on isolated membranes in which the location (external or internal surface or intramembranal) on the membrane must be specified. Perhaps the most exciting studies at this time are those on ganglioside biosynthesis in tumorigenic, virus-transformed cell lines. In these cells the block in biosynthesis has been specifically identified as the step catalyzing the following reaction:

$$\text{UDP-GalNAc} + \text{Ceramide-Glc-Gal(NANA)} \xrightarrow{[\text{transferase}]} \text{Ceramide-Glc-Gal(NANA)-NAcGal} + \text{UDP}$$

Such tumorigenic cells have lost the normal cellular property of contact-inhibition, which may well be correlated with the presence of gangliosides (as prominent constituents of the cell membranes). The exciting aspect of the observations is the fact that in those cells that "revert" to become phenotypically normal, with no tumorigenicity and with normal contact-inhibition, the blocked enzyme activity returns to normal. Yet the virus can still be "rescued" from such cells. Clues to control of ganglioside biosynthesis and to mechanisms for expression or suppression of the viral genome would seem to be provided by this model system. The studies are highly relevant to the functional organization of membranes (a problem of basic interest in this Laboratory) and to factors involved in cancer mechanisms. Moreover such studies provide a valuable capability in the important areas of tissue culture and viral mechanisms.

Finally several of the Laboratory projects carry distinctly clinical implications or orientations. The continuing studies on superfused primate cerebral cortex have emphasized problems of trauma and of ischemia and the associated cerebral edema. A reasonably sophisticated design has permitted essentially continuous monitoring of impaired permeability -- at the blood-brain barrier in terms of leakage from blood to brain of labeled albumin, and at the neural cell membrane in terms of outward leakage of intracellular potassium. The experimental preparations embody many features common to the clinical conditions of trauma (including associated edema) and stroke, and the findings that are emerging appear to have obvious clinical relevance. In the somewhat different context of the sphingolipid storage diseases, several clinical applications have become well established. In addition to the diagnostic procedures for those suspected of being afflicted with any of the half-dozen commoner disorders, two important clinical activities are currently being emphasized: screening for the identification of heterozygous carriers (via enzymic assays on washed leukocytes and skin fibroblasts) and monitoring pregnancies at risk. The latter have provided particularly dramatic examples of the reliability and value of the diagnostic procedures. Amniocenteses carried out in the second trimester have provided fetal cells that upon culture have successfully predicted normal fetuses and also fetuses afflicted with Fabry's disease, Niemann-Pick disease, or Gaucher's disease. Therapeutic abortions carried out on the basis of these tests have confirmed with samples of fetal tissue the correctness of the predictions of afflicted fetuses. These results are surely most compelling examples of the fruition in clinical applications of more than a decade of very complex basic research.

At this time the trend of some of the projects of the Laboratory is strongly in the direction of clinical studies, particularly in terms of trials of enzyme "replacement" therapy. Such trials could involve actual administration of pure enzymes in microspheres or other forms suitable for entrapment by hepatic or splenic reticuloendothelial systems or the treatment of patients by a variant of renal dialysis techniques, in which missing enzymes are fixed on the dialysis membranes to "remove" the sphingolipids circulating through the unit in abnormally elevated concentrations. In anticipation of these trends the senior staff of the Laboratory has been engaged in a study of the appropriate steps needed to add a clinical unit of in-patient beds and clinical staff to the Laboratory's capabilities. A proposal is now in the discussion stage with the Director of Intramural Research, NINDS. At a time in our national health programs when much emphasis is on applications of research findings to clinical problems and the delivery of medical care, these developments and proposals in the program of the Laboratory of Neurochemistry seem to be most appropriate and timely.

Serial No. NDS(1)-61 LNC/AAC 810
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Metabolism of Free and Protein-Bound Amino Acids in Neural Tissues.

Previous Serial Number: Same.

Principal Investigator: Dr. D. B. Tower

Other Investigators: Mr. Oscar Young, Dr. George Allen.

Cooperating Units: Section on Enzyme-Drug Interaction, LCP, NHLI; Section on Myelin Chemistry, LCM, NIMH; Mechanical Engineering Section, BEI, DRS; Karlsen Shipping Co., Ltd. (New Harbour, Nova Scotia, Canada).

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives and Methods: See previous reports.

Major Findings:

(A) Additional characterization of the acidic protein-isolated in pure form from the membrane-rich subfraction of hepatic microsomes was completed. Antibodies prepared against the protein (in rabbits) were used in double diffusion studies in agarose, in agar Ouchterlony plates, and in cellulose acetate membranes. In each case a single arc of precipitation was obtained with concentrations of protein up to 3 mg/ml and separation of wells up to 1 cm (lower limit of detection, 20 μg of protein/ml). The pure protein bound hematin at alkaline pH to give spectral peaks of α_{557}, β_{526} and γ_{423} with molar extinction coefficients (for a mol. wt. of 50000) of 87, 46 and 490, respectively. The reconstituted hemoprotein combined with carbon monoxide. No oxidized hemoprotein could be formed under the conditions employed. In collaboration with Drs. Sollow and Gillette (LCP, NHLI), when antibodies to the protein were added to fresh rat liver microsomes, the initial fast rate of reduction of cytochrome P_{450} with NADH was decreased by 40-50%. On the basis of accumulated evidence, we suspect that our purufied protein could well be the apoprotein of a microsomal cytochrome. Current studies are directed at surveying the species and tissue distribution of the HBMA protein and its response to drug-inducing procedures both with immunochemical techniques.

(B) The opportunity presented to amplify previous comparative neurochemical studies with samples of whole brain from two species of great whales, the fin whale (*B. physalus*) and the sperm whale (*P. catodon*), with brain wts. averaging 6800 to 7800 g, respectively. Field conditions at the Nova Scotia coastal whaling operation precluded fresh biopsies, as originally planned, but samples (∿ 1 kg) of brain were obtained from an 18.3 m fin whale at about 20 hrs. and from a 13.7 m sperm whale at about 15 hrs. after death and frozen in dry ice. Despite the relatively long postmortem intervals, evidence of autolysis was surprisingly minimal. Analyses of lipid composition (by Dr. Trams, LNC), of ganglioside distribution on TLC (by Mr. Bradley and Dr. Brady, LNC), of total cortical proteins (and of the glutamyl, aspartyl and amide moieties thereof), of dry weights, and of electrolytes were all within normal ranges expected from data on other mammalian species. Myelin basic protein, isolated from white matter and electrophoresed on polyacrylamide gel, migrated as a single band characteristic of all but rodent mammalian species (studies by Drs. Martenson and Kies, LCM, NIMH). The lipid, protein, ganglioside and myelin studies indicated no more autolysis than seen in human or beef brains at 1-2 hrs postmortem.

Chloride in whale cerebral cortex averaged 65 uequiv/g, as predicted from the regression curve of Bourke *et al.* (Am. J. Physiol. 208, 682, 1965). Total cholinesterase activity of cerebral cortex averaged 0.78 µmole of acetylthiocholine hydrolyzed/g/min, as predicted from the regression curve of Tower & Elliott (Am. J. Physiol. 168, 747, 1952). Butyryl ChE activity with butyrylthiocholine as substrate and in the presence of a specific inhibitor of AChE was about 10% of the total activity (acetylthiocholine and no inhibitor) and was not significantly different from BuChE activities measured in rat, cat and beef cerebral cortex. Oxygen consumption of cerebral cortical slices in phosphate-buffered medium (3 mM K) averaged 21 µmoles/g/h, as predicted from the regression curve for frozen and thawed slices obtained for rat, cat and beef cerebral cortex (consistently at 48-99% of the values reported for fresh, unfrozen slices by Elliott and Henderson in J. Neurophysiol. 11, 473, 1948).

The establishment of these points for whale cerebral cortex on the respective interspecies regression curves permit more accurate predictions of the normal values for human cerebral cortex, namely, 59 uequiv of Cl^-/g; 1.03 µmoles of ACh hydrolyzed/g/min; and 80 µmoles of O_2 uptake/g/h. The data for BuChE provide additional support for our previous contention that the density of glial cells in cerebral cortex remains relatively constant over the range of species from rodents to whales. The preservation of oxidative metabolism in 15-20 h postmortem whale brains, frozen en bloc in dry ice under crude field conditions, was most surprising but was duplicated with beef brains treated in similar fashion. Since oxygen consumption is not preserved in frozen and thawed slices of cortex unless great precautions are taken to process them slowly in graded concentrations of glycerol, our experiences with the en bloc freezing of large brains is intriguing. Further studies of this and related cryogenic phenomena are in progress.

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To continue studies along the lines indicated.

Publications:

1. Wherrett, J. R. and Tower, D. B.: Glutamyl, aspartyl and amide moieties of cerebral proteins: metabolic aspects *in vitro*. *J. Neurochem.*, in press.

2. Tower, D. B. and Wherrett, J. R.: Glutamyl,and aspartyl moieties of cerebral proteins: enrichment in membrane-containing microsomal subfractions. *J. Neurochem.*, in press.

3. Tower, D. B. (Editor): *Neurochemistry in the Soviet Union*, Bethesda, National Institutes of Health, 1970, ii + 91 pp.

4. Tower, D. B.: Johann Thomas Hensing (1683-1726). In: Haymaker, W. and Schiller, F. (Eds.): *The Founders of Neurology* (2nd ed.), Springfield, CC Thomas, 1970, pp. 285-289.

5. Tower, D. B.: Johann Ludwig Wilhelm Thudichum (1829-1901). In: Haymaker, W. and Schiller, F. (Eds.): *The Founders of Neurology* (2nd ed.), Springfield, CC Thomas, 1970, pp. 297-302.

6. Tower, D. B.: Nicolas-Louis Vauquelin (1763-1829). In: Haymaker, W. and Schiller, F. (Eds.): *The Founders of Neurology* (2nd ed.), Springfield, CC Thomas, 1970, pp. 302-306.

Serial No. NDS(1)-61 LNC/AAC 811
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrolytes and Energy Metabolism in Cerebral Tissues.

Previous Serial Number: Same.

Principal Investigators: Drs. D. B. Tower, F. A. Matsen and C. R. West.

Other Investigators: Mr. Oscar Young.

Cooperating Units: Surgical Neurology Branch, IR, NINDS; Department of Neurosurgery, Roswell Park Memorial Institute, Buffalo, New York.

Man Years:

Total:	2.5
Professional:	2.5
Other:	0

Project Description:

Objectives and Methods: See previous reports.

Major Findings:

(A) Modifications of the previously reported procedure for perfusion over the surface of the monkey (*M. mulatta*) cerebral cortex *in vivo* were developed to simplify the preparative phases and to minimize the complications and morbidity. Pairs of 0.8 mm - inside-diameter stainless steel ports 3 cm apart are placed bilaterally through small craniotomy openings and are fixed to the bone, with footplates inserted through dural slits. The system does not leak unless perfusion pressures exceed 25 mm of Hg; usual pressures are 5 mm of Hg at a perfusion rate for artificial CSF of 0.077 ml/min. In labelling experiments with $[^{131}I]$ serum albumin and $[^{14}C]$ inulin essentially no solute exchange between the left and right perfused areas occurred (concentration ratios of 2000:1). In monkeys injected intravenously with chromatographed $[^{131}I]$ albumin, the rate of passage of albumin from the vascular compartment into the perfusion area averaged over a 9-hr period 2.7 μg/min per cm^2 of cortical surface area.

(B) The preparation as already described has been used to evaluated changes in fluids and electrolytes in two experimental conditions, a unilateral local cold-lesion (equivalent to a traumatic lesion) and unilateral internal carotid ligation (after isolation from external carotid and communicating arteries). On the side of the cold lesion, the rate of entry of labelled albumin from the blood rose 3-fold within 45 min after making the lesion and persisted at twice the rate of entry into the contralateral control area for over 3 hours. Within the initial 30 minutes after carotid ligation the concentration of K^+ in the cortical effluent had risen significantly ($P = 0.01$) above the preligation homolateral and simultaneous contralateral control concentrations to maximal levels at 90-120 min after ligation 15% above preligation homolateral and simultaneous contralateral control concentrations. Significantly elevated concentrations of K^+ in cortical effluents from the experimental hemisphere persisted for at least 6 hours after removal of the ligature from the carotid artery. The studies are still being actively pursued and more complete data will become available subsequently. The currently available data indicate that the present perfusion procedure can provide extremely valuable information on acute changes that occur in association with traumatic and vascular insults to the cerebral gray matter. Eventual correlation of these data with those on edema and the role of chloride reported previously and currently being continued by Dr. Bourke (now in Buffalo) should provide a comprehensive picture of fluid and electrolyte dynamics in experimental models for cerebral edema, cerebral trauma and cerebral ischemia.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged functions of hyperactivity states.

Proposed Course: To continue investigations along lines indicated by previous results.

Honors and Awards:

Tower, D. B.:

William G. Lennox Guest Lecturer at the Annual Meeting of the American Epilepsy Society, December 1970, New York City.

Publications:

1. Tower, D. B.: Hans Winterstein (1879-1963). In: Haymaker, W. and Schiller, F. (Eds.): The Founders of Neurology (2nd ed.), Springfield, CC Thomas, 1970, pp. 307-311.

2. Tower, D. B.: Fluids and electrolytes in the central nervous system: factors affecting their distribution, with special reference to excitability and edema. Electroenceph. clin. Neurophysiol., Suppl., in press.

3. Tower, D. B.: Cerebral edema. In: Albers, R. W., Agranoff, B. W., Katzman, R. and Siegel, G. J. (Eds.): Basic Neurochemistry, Boston, Little, Brown & Co., in press.

Serial No. NDS(1)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: Dr. R. W. Albers.

Other Investigators: Drs. R. Collins, S. Goldman, D. Jean; and Mr. G. J. Koval.

Cooperating Units: Section on Trophic Nerve Function, LNNS, NINDS.

Man Years:

Total:	5.0
Professional:	4.0
Other:	1.0

Project Description:

Objectives and Methods: See previous reports.

Major Findings:

(a) Completion of a study of the relation of the K^+-activated phosphatase to the Na^+-K^+-activated ATPase: Substrates for the K^+-phosphatase appear to be acted upon by a catalytic site for which the normal substrate is the phosphoryl protein moiety of the ATPase. (Koval & Albers)

(b) Completion of a kinetic analysis of the effect of Na^+ ion on the K^+ activation of the phosphatase: Multiple activation sites are indicated for the K^+-activation, all but one of which may be replaced by Na^+. (Koval & Albers)

(c) Characterization of the phosphorylated peptide of the ATPase has progressed to the point that work on its amino acid composition is now in progress. (Collins)

(d) Exploration of the role of phospholipids in the Na$^+$-ATPase system supports the concept that separate moieties of the enzyme system are concerned with protein phosphokinase and protein phosphatase activities, respectively. (Goldman)

(e) Continuation of the collaborative myosin project is reported by the Section on Trophic Nerve Function, LNNS (Serial No. NDS(1)-63 LNNS/TNF 1054). (Jean)

Significance: See previous reports.

Proposed Course: Some aspects in each of the reported studies need further exploration, but emphasis of the program is expected to shift from kinetic to structural studies on both the myosin and Na$^+$-K$^+$-ATPase systems.

Publications:

1. Albers, R. W. and Siegel, G. J.: "Nucleotide phosphohydrolases," chapter in "Handbook of Neurochemistry,". In Lajtha, A. (Ed.): Plenum Press, New York, Vol. 4, 1970, pp. 13-44.

2. Samaha, F. J., Guth, L. and Albers, R. W.: The neural regulation of gene expression in the muscle cell. *Exp. Neurol.* 27: 276-282, 1970.

Serial No. NDS(1)-61 LNC/LC 815
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Metabolism of Complex Lipids of Nervous Tissue. Studies on Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease, Krabbe's Disease and Metachromatic Leukodystrophy.

Previous Serial Number: Same.

Principal Investigator: Dr. Roscoe O. Brady.

Other Investigators: Drs. A. E. Gal, W. G. Johnson, Mr. R. M. Bradley, and Mr. G. E. Mook.

Cooperating Units: Weizmann Institute of Science, Rehovoth, Israel.

Man Years:

Total:	3.0
Professional:	2.8
Other:	0.2

Project Description:

Objectives and Methods: See previous reports.

Major Findings: Identifications of heterozygous carriers of the Gaucher's, Niemann-Pick and Fabry's disease respective traits are now established through refinements of assay procedures for the respective involved enzymes in extracts of washed leukocytes and skin fibroblasts. The U. S. Government has been assigned the rights for the now-patented leukocyte diagnostic test, developed in this laboratory, for detecting patients with these diseases.

We have obtained highly enriched preparations of the glucocerebrosidase defective in Gaucher's disease by means of column chromatographic purification procedures, and even more impressive results have been obtained in the purification of the alpha-galactosidase defective in Fabry's disease.

Significance: Our diagnostic skills have improved to the extent that we have monitored confidently pregnancies at risk for Gaucher's disease, Niemann-Pick disease and Fabry's disease. With these procedures we have accurately identified fetuses with Fabry's disease, Niemann-Pick disease and Gaucher's disease in the second trimester.

Proposed Course: Continuation of work on purification of enzymes involved in the metabolic disorder in Gaucher's disease and in Fabry's disease, with the hope of soon obtaining sufficient quantities of enzyme for trials of replacement therapy. Continued monitoring of pregnancies at risk in lipid storage diseases and initiation of a screening program for identification of heterozygous carriers.

Honors and Awards:

Brady, R. O. G. Burroughs Mider NIH Annual Lectureship Award December 16, 1970.

Brady, R. O. American Chemical Society Chemical Innovator Selection, February 22, 1971.

Publications:

1. Brady, R. O.: Enzymic defects in lipid storage diseases. Biochem. J. (London) 117: 8-10, March 1970.
2. Brady, R. O.: Cerebral Lipidoses. Ann. Rev. Med., Vol. 21: 317-334, June 1970.
3. Brady, R. O.: Biochemical approaches to the nosology of nervous system defects. In McKusick, V. A. (Ed.): The Clinical Delineation of Birth Defects, New York, The National Foundation, 1971, pp. 33-36.
4. Brady, R. O.: Lipidoses. In Nitowsky, H. M. (Ed.): Chapter in Barnett's Pediatrics, New York, Appleton Century Crofts, 1971.
5. Brady, R. O.: Metabolic disorders of sphingolipid metabolism in man. Chem. Phys. Lipids, 5: 261-269, August 1970.
6. Brady, R. O.: Prenatal diagnosis of lipid storage diseases. Clin. Chem., 16:811-815, September 1970.
7. Austin, J., Suzuki, K., Armstrong, D., Brady, R., Bachhawat, B. K., Schlenker, J., and Stumpf, D.: Studies in globoid (Krabbe) leukodystrophy (GLD) V. Controlled enzymic studies in ten human cases. Arch. Neurol., 23: 502-512, December 1970.
8. Brady, R. O.: The genetic mismanagement of complex lipid metabolism. Bull. N. Y. Acad. Med., 47: 173-182, February 1971.
9. Ziboh, V. A., and Brady, R. O.: Inhibition of lipid synthesis in rat skin by tetrolyl-pantetheine. Proc. Soc. Exp. Biol. Med., 136: 15-18, January 1971.

10. Brady, R. O.: The ups and downs of complex lipid metabolism. in Bernsohn, J. (Ed.): *Lipid Storage Diseases*, New York, N. Y., Academic Press, March 1971, pp. 275-289.

11. Brady, R. O., Uhlendorf, B. W., and Jacobson, C. B.: Fabry's disease: prenatal detection. *Science* 172: 174-175, April 1971.

12. Brady, R. O., Johnson, W. G., and Uhlendorf, B. W.: Identification of heterozygous carriers of lipid storage diseases. Current status and clinical applications. *Amer. J. Med.*, in press.

13. Epstein, C. J., Brady, R. O., Schneider, E. L., Bradley, R. M., and Shapiro, D.: *In utero* diagnosis of Niemann-Pick disease. *Am. J. Hum. Genet.*, in press.

U. S. Patent No. 3,531,374

Brady, R. O., and Kampine, J. P.: Diagnostic test for Gaucher's disease and Niemann-Pick disease. Issued September 29, 1970, assigned to the United States of America.

Serial No. NDS(1)-61 LNC/LC 816
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Dr. A. E. Gal, Mr. John Tallman, and Miss J. M. Quirk.

Cooperating Units: None.

Man Years:

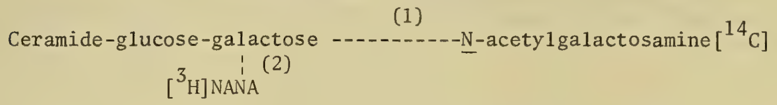
Total:	2.5
Professional:	2.4
Other:	0.1

Project Description:

Objectives and Methods: See previous reports.

Major Findings: The site of the metabolic defect in Tay-Sachs disease has now been conclusively identified with the use of Tay-Sachs ganglioside specifically labeled in the N-acetylgalactosaminyl portion of the molecule. There is complete absence of the enzyme which catalyzes the cleavage of the terminal molecule of N-Ac-Gal from Tay-Sachs ganglioside (Site 1) in the brain tissue of patients with the disease (previously reported data were on muscle and hence not conclusive):

TAY-SACHS GANGLIOSIDE



$$\underset{\substack{\vdots\ (2)\\ [^{3}\text{H}]\text{NANA}}}{\text{Ceramide-glucose-galactose}}\ \overset{(1)}{\text{-----------}}\ \underline{\text{N}}\text{-acetylgalactosamine}[^{14}\text{C}]$$

The deficiency contrasts with the normal levels in these patients of the enzyme which catalyzes cleavage of the N-acetylneuraminic acid (NANA) moiety from the ganglioside (Site 2); as demonstrated for gangliosides specifically labeled in the respective portions of the molecule.

Significance: The demonstration of the nature of the metabolic defect in Tay-Sachs disease now permits the consideration of rational therapeutic measures for attempts at alleviating this disorder. Furthermore, tests have been developed for the early diagnosis of infants as well as the antenatal detection of fetuses affected with Tay-Sachs disease.

Proposed Course: We shall extend our work on the purification of the enzymes involved in the catabolism of Tay-Sachs ganglioside. In particular, we firmly believe we shall find the solution to the problem of why the entire molecule of Tay-Sachs ganglioside accumulates in the neuronal cells of patients with this disease in the presence of normal levels of Tay-Sachs ganglioside neuraminidase. Based on this observation, one would initially expect an accumulation of the corresponding ceramide-trihexoside (Tay-Sachs ganglioside minus the N-acetylneuraminic acid moiety). This point will be resolved and an explanation will be provided for the lack of systemic organomegaly in Tay-Sachs disease contrasted with all of the other lipid storage disorders.

Publications:

1. Brady, R. O., and Kolodny, E. H.: Disorders of ganglioside metabolism. In Steinberg, A. G., and Bearn, A. G., (Eds.) Progress in Medical Genetics. New York, N. Y. Grune and Stratton, in press.

2. Max, S. R., Nelson, P. G., and Brady, R. O.: The effect of denervation on the composition of muscle gangliosides. J. Neurochem. 17: 1517-1520, October 1970.

3. Kolodny, E. H., Kanfer, J. N., Quirk, J. M., and Brady, R. O.: Properties of a particle-bound enzyme from rat intestine that cleaves sialic acid from Tay-Sachs ganglioside. J. Biol. Chem. 246: 1426-1431, March, 1971.

4. Tallman, J. F., Brady, R. O., and Suzuki, K.: Enzymatic activities associated with membranous cytoplasmic bodies and isolated brain lysosomes. J. Neurochem. (in press).

Serial No. NDS(1)-61 LNC/LC 817
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunochemical Studies in Multiple Sclerosis.

Previous Serial Number: Same.

Principal Investigator: Dr. Rolf Sammeck.

Other Investigators: Dr. R. O. Brady.

Cooperating Units: None.

Man Years:

Total:	1.3
Professional:	1.1
Other:	0.2

Project Description:

Objectives and Methods: See previous reports.

Major Findings: Turnover studies on myelin basic protein isolated from rats at various ages after intracisternal injection of [^{14}C]tryptophan demonstrate an unexpectedly high rate of renewal of the myelin basic protein in the CNS of mature rats. This finding further increases the likelihood of a role for the myelin basic protein in the etiology of human demyelinating disorders.

Significance: See previous reports.

Proposed Course: Characterization of nature and antigenicity of breakdown products of myelin basic protein and assessment of the findings in terms of relevance to the pathogenesis of multiple sclerosis and related conditions.

Publications:

1. Sammeck, R., Martenson, R. E., and Brady, R. O.: Studies of the metabolism of myelin basic protein in various regions of the central nervous system. Brain Research, in press.

Serial No. NDS(1)-66 LNC/LC 1309
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Metabolism of Sphingolipids in Tumor Tissues.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Dr. F. Cumar, Dr. P. H. Fishman, and Mr. R. M. Bradley.

Cooperating Units: National Cancer Institute.

Man Years:

Total:	2.6
Professional:	2.5
Other:	0.1

Project Description:

Objectives and Methods: See previous reports.

Major Findings: The dramatic difference (described in the 1970 report) between tumorigenic, virus-transformed cell lines and the parent cell lines in the ganglioside composition has now been conclusively characterized as a block of the enzyme which catalyzes an obligatory step in the biosynthesis of gangliosides. In SV-40- and polyoma-virus-transformed cells there is a drastic reduction in the activity of the amino-sugar transferase that catalyzes the transfer of N-acetylgalactosamine from UDP-N-Ac-Gal to hematoside (Ceramide-Glc-Gal-NANA) to form Tay-Sachs ganglioside, a normal tissue component. In certain "flat revertant" cells which had been previously transformed with virus, the activity of the blocked enzyme returns to normal. This restoration of enzymatic activity occurs in cells from which the virus can be "rescued". However, these revertant cells are phenotypically normal, exhibit contact-inhibition in culture, and are no longer tumorigenic .

Significance: These experiments indicate that there are fundamental changes in the metabolism and composition of sphingolipids in tumorigenic virus transformed cells. These findings are of considerable relevance to the possible role played by these membrane lipids in the loss of contact inhibition exhibited by transformed cells. Elucidation of the control processes which bring about these metabolic alterations is potentially very important for obtaining insight into metabolic changes that occur in oncogenesis.

Proposed Course: Extension of studies to RNA-virus transformed cells and to cells rendered tumorigenic as the chemical carcinogens. Elucidation of mechanism(s) by which presence of the viral genome is or is not expressed and the mechanism(s) for the consequent control of ganglioside biosynthesis.

Publications:

1. Cumar, F. A., Brady, R. O., Kolodny, E. H., McFarland, V. W., and Mora, P. T.: Enzymatic block in the synthesis of gangliosides in DNA virus-transformed tumorigenic mouse cell lines. *Proc. Nat. Acad. Sci. U. S.* 67: 757-764, October 1970.

2. Brady, R. O., and Cumar, F. A.: Alteration in ganglioside pattern and synthesis in SV 40 and polyoma virus-transformed mouse cell lines. *Biochim. Biophys. Acta* 218: 309-319, November 1970.

3. Mora, P. T., Brady, R. O., and Smith, R. W.: Glycolipids in SV 40 and polyoma virus transformed mouse cell lines. In: D. Aminoff (Ed.) *Blood and Tissue Antigens*. New York, N. Y., Academic Press, July 1970, pp. 337-340.

4. Cumar, F. A., Fishman, P. A., and Brady, R. O.: Analogous reactions for the biosynthesis of monosialo- and disialo-gangliosides in brain. *J. Biol. Chem.*, in press.

5. Mora, P. T., Cumar, F. A., and Brady, R. O.: A common biochemical change in SV40 and polyoma virus transformed mouse cells coupled to control of cell growth in culture. *Virology*, in press.

Serial No. NDS(1)-67 LNC/LC 1457
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: Same.

Principal Investigator: Dr. A. E. Gal.

Other Investigators: Mr. F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	1.7
Professional:	1.6
Other:	0.1

Project Description:

Objectives: See previous reports.

Methods and Major Findings:

Two approaches are used in labelling the glycolipids: Non-specific and specific labelling.

The technique of the Wilzbach gas exposure is used for the labelling of the sphingolipids non specifically. In using this method the lipids are treated with tritium under optimal conditions of pressure, temperature, and time. As an example ceramide trihexoside (the lipid which accumulates in patients having Fabry's disease) has been tritiated and then purified by multistep procedures to eliminate isomers, breakdown products and other impurities.

Specific labelling is under study with aminosugars and with neuraminic acid. In this method a functional group (carboxy or acetyl) is labelled by exchange reaction between a labelled and a non radioactive one. This unique approach is well suited to the labelling of organic molecules with ^{3}H or ^{14}C, particularly in cases when the compound cannot be obtained by synthesis. On the basis of the methods which we have developed with the above-mentioned saccharides, work is in progress on the labelling of the different types of gangliosides.

Significance: See previous reports.

Proposed Course: Non specific labelling of various glycolipids and the labelling by exchange reactions will be pursued. The optional conditions for synthesizing neuraminic acids by the exchange of acetyl groups will be studied further. The technique will then be applied to more complex neuraminic acid derivatives and finally the method will be used for the preparation of specifically labelled gangliosides.

Besides the great importance of solving structural and structure-activity problems and the use as a diagnostic tool in a research setting, these compounds could then be produced in larger quantities and with sufficiently high specific activities such that their use could probably be extended to use as routine clinical diagnostic procedures.

Publications:

1. Brady, R. O., O'Brien, J. S., Bradley, R. M., and Gal, A. E.: Sphingolipid hydrolases in brain tissue of patients with generalized gangliosidosis. Biochim. Biophys. Acta, 210: 193-195, 1970.

Serial No. NDS(1)-68 LNC/LC 1550
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Myelination in the Central Nervous System.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Mr. Roy M. Bradley, Dr. Donald Silberberg

Cooperating Units: Department of Neurology, University of Pennsylvania School of Medicine.

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description:

Objectives: See previous reports.

Methods: Procedures are being developed for obtaining myelinating explants of neonatal tissue. The effects of selected enzymes and enzyme inhibitors on the myelination process are being examined.

Major Findings: The addition of partially purified neuraminidase preparations has a pronounced effect on the gangliosides of myelinating explants, whereas there was no detectable effect on the time course or extent of myelination in tissue cultures.

Significance: Our procedures should permit examination of the role and contribution of various types of cells and enzymes for the formation and maintenance of the myelin sheath. This information is of particular importance for investigating biochemical abnormalities in myelinopathic conditions such as multiple sclerosis and hereditary demyelinating diseases.

Proposed Course: Our investigations will be extended to include a comparative study of the composition and metabolism of individual cell lines obtained from human and animal brain sources. The effects of specific inhibitors and activators of potentially involved enzymes will be investigated.

Publications: None.

Serial No. NDS(1)-70 LNC/LC 1808
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Glycoprotein Metabolism in the Myelinating Central Nervous System.

Previous Serial Number: None.

Principal Investigator: Dr. R. H. Quarles.

Other Investigators: Dr. R. O. Brady; Mr. M. S. Glasgow

Cooperating Units: None.

Man Years:

Total:	2.4
Professional:	2.2
Other:	0.2

Project Description:

Objectives: To investigate the biochemistry of the surfaces of cells of the nervous system with particular regard to glycoprotein components.

Methods: Specific radioactive precursors of glycoproteins are used to study the metabolism of glycoproteins in developing rat brain. The membrane-bound glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Major Findings: Radioactive fucose is an efficient and specific precursor of brain glycoproteins *in vivo* and *in vitro*. Incorporation of fucose into glycoproteins is most rapid at birth and decreases with increasing age. Upon polyacrylamide gel electrophoresis the radioactive glycoproteins are very heterogeneous in terms of molecular weight. Development of a double labeling procedure using fucose-[^{3}H] and fucose-[^{14}C] together has permitted us to demonstrate that the incorporation of radioactive fucose into the high-molecular-weight glycoproteins is relatively greater in the brains of 5-day-old rats than in those of 25-day-old rats. By intracerebral injection of N-acetyl-[^{3}H]-mannosamine a high degree of specificity is obtained for the labeling of the sialic acid moieties in glycoproteins and gangliosides. In rats injected with a mixture of fucose-[^{14}C] and N-acetyl-[^{3}H]-mannosamine there are differences in the relative degrees of incorporation of the two radioactive precursors into the various glycoproteins, with the greatest incorporation of fucose-[^{14}C] relative to that of N-acetyl-[^{3}H]-mannosamine in some of the smaller-molecular-weight glycoproteins.

Significance: During maturation of the nervous system many new intercellular connections become established, and specific cell-cell recognition factors must play an immensely important role in the establishment of these relationships. Since glycoproteins are well known components of cell surfaces, it is quite likely that specific alterations of these molecules contribute to the developmental process. The glycoproteins which are synthesized preferentially in the brains of very young animals may be involved in the rapid proliferation of neuronal and glial processes that occurs at that time. Fucose and sialic acid are prime candidates to affect cellular interactions because of their different physical properties and their terminal positions on glycoproteins. Thus the radioactive precursors which we are using should prove very valuable in studying the metabolism of these terminal sugars and their possible role in cellular interactions.

Proposed Course: The use of radioactive precursors and polyacrylamide gel electrophoresis makes it possible to investigate glycoproteins and their metabolism in very small tissue samples (<5 mg wet weight). Therefore we propose to study glycoprotein metabolism in brain samples of defined anatomical structure, both in terms of particular regions of the nervous system and in terms of purified subcellular fractions (myelin, synaptasomes, etc.). This approach will probably permit the identification of individual glycoproteins which are involved in specified cellular interactions.

Publications:

1. Quarles, R. H., and Brady, R. O.: Sialoglycoproteins and several glycosidases in developing rat brain. *J. Neurochem.* 17: 801-807, 1970.

2. Quarles, R. H., and Brady, R. O.: Synthesis of glycoproteins and gangliosides in developing rat brain. *J. Neurochem.*, in press.

Serial No. NDS(1)-67 LNC/PM 1480
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. D. P. Rall; Dr. E. A. Brown, Dr. D. B. Tower; Dr. F. Snyder; Prof. A. A. Benson

Cooperating Units: Experimental Therapeutics, NCI; Laboratory of Chemical Pharmacology, NHLI; Laboratory of Clinical Psychobiology, NIMH; Mote Marine Laboratory (Sarasota, Fla.); Scripps Institute of Oceanography (La Jolla, Calif.); National Air Pollution Administration (Cincinnati, Ohio).

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives and Methods: See previous reports.

Major Findings:

(a) Current studies on the metabolism of substituted quinolines have been extended to the comparative pharmacology of this class of compounds. Methyl substituted quinolines appear to pass the blood-brain barrier rather readily, and distribution studies indicated a preferential uptake of 2-methyl quinoline by brain tissue in mammals (mice), as well as in elasmobranchi and teleosts. With some limited success we have followed drug uptake and depth of anesthesia in sharks and rays by EEG. In collaboration with Dr. F. Snyder (Chief, LCP-NIMH) intracerebral electrodes were inserted into nurse sharks and cow nose rays; the signals were recorded either by hard wire or by radio telemetry. Subsequently the animals were exposed to known concentrations of quinaldine. Such technology proved to be feasible and susceptible to a number of important applications. Our relative inexperience in terms of the electrical output of elasmobranch CNS did not permit definitive correlations between EEG changes and drug levels.

(b) In collaboration with Dr. D. B. Tower (Chief, LNC-NINDS) we have studied the lipid composition of gray matter and white matter from two species of cetacean great whales (*B. Physalus* and *P. catodon*) and and compared their compositions to the lipid composition of an elasmobranch. The levels of neutral lipids, cholesterol, sphingolipids and phospholipids in gray and white matter were determined by column chromatography and TLC. From a comparative neurochemical viewpoint, the lipid composition of cetacean or selachian brains does not differ substantially from that reported for a wide variety of other, mostly mammalian species.

(c) Because of our prior experience with lipid synthesis in various species, we were invited to collaborate with Prof. A. A. Benson (Scripps Institution of Oceanography) in an investigation of the biosynthetic pathways leading to wax ester formation in marine copepods. Wax ester biosynthesis in small marine crustaceans may be of great ecological importance, since it has been estimated that about one-half of the ocean's photosynthetic products are converted into wax esters when they pass through the crustacean food chain. Working with enzymes prepared from *Calanus* and *Gaussia* we were able to show that wax ester synthesis proceeded according to the following scheme:

$$(1) \quad CH_3\ (CH_2)_nCOOH \xrightarrow{NADH} CH_3(CH_2)_nCH_2OH + NAD$$

$$(2) \quad CH_3\ (CH_2)_n\ CH_2OH + CH_3(CH_2)_nCOSCoA \longrightarrow$$

$$CH_3(CH_2)_n\ COOCH_2(CH_2)_nCH_3 + CoA$$

In some further experiments we obtained evidence that the conversion of free fatty acids (FFA) to the respective aldehydes and alcohols, (by reduction with NADH), probably proceeded through the coenzyme A derivatives. Similarly, esterification of the long chain alcohol with FFA required the CoA derivative.

(d) We received a request from the National Air Pollution Control Administration (Cincinnati, Ohio) to analyze brain biopsy specimen from several series of dogs which had been exposed for 18 months to low levels of ozone (O_3). The question posed was whether the observed physiological changes could be correlated with neurochemical alterations. In collaboration with Dr. E. A. Brown (LCP-NHLI) and Mr. O. Young, (LNC-NINDS), we carried out the following analyses in control and ozone-exposed dog brains: Na^+, K^+ and Cl^- content; content of lipid hydroperoxides; content of epinephrine and nor-epinephrine; activity of Mg^{2+}, Na K ATPase, acetylcholinesterase, monoamineoxidase (MAO) and catechol-O-methyltransferase (COMT), all by established methods. We concluded that slight, but statistically significant alterations of catecholamine metabolism had resulted from the chronic exposure to O_3. In particular, we found changes in MAO and COMT activity, with some apparent correlation between the degree (or extent) of exposure and the observed changes in enzymatic activity.

Significance: Emphasis is placed on the interdisciplinary aspects of these studies. Contributions to a variety of studies have been possible because the skill and the background of the LNC personnel has developed into a widely-accepted resource in neurochemical research. In consequence, although the primary objectives of this project continue to be a comparative study of neurotransmitter metabolism in marine animals, some of these studies also represent contributions to investigations concerned with the state (or control) of our environment.

Proposed course: The Section on Physiology and Metabolism is committed to further participation in such collaborative studies, including our studies on comparative aspects of neurotransmitter metabolism.

Honors and Awards: E. G. Trams; Guest Lecturer on Comparative Biochemistry, University of California, San Diego, April-May 1970; The Pennsylvania State University; October 1970.

Publications:

1. Trams, E.G., and Hoiberg, C.P.: Lipid Composition of tissues from *Electrophorus electricus*. *Proc. Soc. Exp. Biol. and Med.* 135: 193-196, Oct. 1970.

Serial No. NDS(1)-67 LNC/PM 1481
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Composition and Metabolism of Isolated Cellular Membranes.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. A. Solyom; Mr. Carl J. Lauter; Prof. S. Patton.

Cooperating Units: Div. Food Sci., Pennsylvania State University.

Man Years:

Total:	2.8
Professional:	2.8
Other:	0

Project Description:

Objectives: See previous reports.

Methods: The primary source for plasma membranes has been the hepatic parenchymal cell. A variety of methods for the isolation of plasma cell membranes from a number of species were compared. Our current experimental design requires the preparation of plasma cell membranes from isolated liver cells. Consequently we had to establish a new technology for the preparation of isolated liver cells. Currently, we perfuse exteriorized livers from rats or guinea pigs with collagenase and hyaluronidase. After the perfusion, the liver tissue is pressed through wide mesh screens and the resulting mixture is subjected to further enzymatic attack. The isolated liver cells are separated from cell debris and studied directly or subjected to a preparative procedure for isolation of membranes. Studies of viability and metabolic experiments are conducted on the isolated cells or the membranes by a wide range of analytical techniques.

Major Findings:

(A) Conclusion of the study on metabolism of fatty acyl coenzyme A by plasma membranes. The kinetics of many of the enzymes in the plasma membrane were studied under various conditions. Some of the major reactions which have been investigated are summarized as follows:

(1) The initial reaction in the catabolism of CoA involved the action of a nucleotide pyrophosphatase (EC 3.6.1.9). The products expected from this reaction are 3',5'-ADP and 4'-phospho-S-palmitylpantetheine.

$$\text{Palmityl-CoA} \xrightarrow{\text{Nucleotide pyrophosphatase}} \text{3',5'-diphosphoadenosine} + \text{4'-phospho-S-palmitylpantetheine}$$

When the initial concentration of palmityl-CoA in an incubation mixture at pH 7.4 was above critical micellar concentration, Lineweaver-Burk plots gave a K_m of 0.56 mM and a value for v_{max} of 10 nmoles/min per mg membrane protein. At concentrations below the critical micellar concentration, however, we found a K_m of 8.0-9.3 μM and a v_{max} of 42-50 nmoles/min per mg.

The highest activity of nucleotide pyrophosphatase was observed with NADH, where at pH 9.5, v_{max} = 1.1 μmoles/min per mg, and at pH 7.5, v_{max} = 180 nmoles per min per mg. K_m and v_{max} with FAD at pH 9.0 were 0.67 mM and 200 nmoles/mg per min, respectively.

(2) The major metabolic product derived from the pantothenyl moiety of palmityl-CoA was S-palmitylpantetheine. The dephosphorylation of 4'-phosphopantetheine, or its acyl derivative, was catalyzed by membrane-bound phosphomonoesterase (EC 3.1.3.1).

$$\text{4'-Phospho-S-acylpantetheine} \longrightarrow \text{acylpantetheine} + P_i$$

(3) Since 3',5'-ADP was initially dephosphorylated to yield 3'-AMP and P_i, we considered the possibility that the 5' phosphate was removed by the action of 5'-nucleotidase. It could be shown, however, that 3',5'-ADP did not competitively inhibit the dephosphorylation of 5'-AMP. In contrast, 3',5'-ADP was found to be an excellent competitive inhibitor of the dephosphorylation of p-nitrophenyl phosphate. At pH 7.4 the K_i for 3',5'-ADP was 0.68 mM and at pH 8.5, 0.024 mM. We concluded that this reaction was catalyzed by a non-specific phosphomonoesterase.

$$\text{3',5'-ADP} \xrightarrow{\text{phosphomonoesterase}} \text{3'-AMP} + P_i$$

3'-AMP was formed during incubation with CoA or 3',5'-ADP but did not accumulate in substantial amounts. The possibility was considered that the reaction

$$\text{3'-AMP} \longrightarrow \text{adenosine} + P_i$$

was catalyzed by a non-specific phosphomonoesterase. Kinetic studies revealed that 3'-AMP was a competitive inhibitor for p-nitrophenyl phosphate (at pH 7.4, K_i = 1.83 mM). In the presence of 1 mM Mg^{2+} the K_m for 3'-AMP was 31 μM at pH 8.9 and v_{max} = 15.1 nmoles/mg per min.

(4) 5'-nucleotidase (EC 3.1.3.5) has been previously described as a plasma membrane marker enzyme, and in our preparations it has a specific activity of 0.97 μmoles of adenosine liberated per min per mg membrane protein. A K_m of 30 μM was determined at pH 7.4. The pH optimum was around 9.0 and at that pH, 10 mM Mg^{2+} produced a 50% stimulation with EDTA-dialyzed membranes.

$$\text{5'-AMP} \longrightarrow \text{adenosine} + P_i$$

The addition of 50 μM Co^{2+} at pH 7.4 and at pH 9.4 produced a 90 and 30% stimulation, respectively. Addition of Zn^{2+} above 50 μM proved to be inhibitory.

(5) At least three different ATPase activities were found in our membrane preparations. Incubation of membranes obtained by the procedure of Neville with [γ-^{32}P] ATP showed that a very active Mg^{2+}-dependent ATPase was present.

$$\text{Adenosine-P-P-}^{32}P \xleftrightarrow[Mg^{2+}]{\text{ATPase}} \text{ADP} + {}^{32}P_i$$

Comparision of the P_i liberated with the ^{32}P data provided an estimate for the activity of ATP pyrophosphohydrolase

$$\text{Adenosine-P-P-}^{32}P \xleftrightarrow{\text{ATP pyrophosphohydrolase}} \text{AMP} + \text{P-}^{32}P$$

The pH optimum of this enzyme appeared to be at 10.5 (sodium glycinate buffer) and its properties appeared to be closely related to those described in the literature.

(6) Cleavage of the thioester bond of S-acylpantetheine was catalyzed by a thioester hydrolase (palmityl-CoA hydrolase (?), EC 3.1.2.2)

$$\text{Palmityl-CoA} \xrightarrow{\text{thioesterase}} \text{palmitic acid} + \text{CoA}$$

The thioester hydrolase was most active with the $C_{18:0}$ fatty acid substituent, as previously described. The pH optimum for the enzyme was about 9.0 and the esterase was inhibited by the addition of 1 mM phenylmethylsulfonyl fluoride.

(7) A P_i deficit was consistently observed with a variety of substrates. This deficit was greatest during short-term incubations and tended to disappear when the incubation time was extended to 1 or 2 h. We assume that this P_i deficit was due to a phosphotransferase reaction involving a phosphorylation of enzyme protein or lipid, i.e.:

$$\text{3',5'-ADP} + \text{E} \longleftrightarrow \text{3'-AMP} + \text{E-P}$$

(B) We have more recently compared the activity of many of these enzymes in plasma membrane preparations obtained from cat, rabbit and guinea pig to supplement our previous data on the rat. Substantial variations were observed among species. In the case of the 5'-nucleotidase, for instance, if the specific activity in rat liver plasma membranes were taken to equal 100, the relative specific activities for guinea pig, cat and rabbit were 28, 2.3 and 2.2, respectively. We observed similar differences for the nucleotide pyrophosphatases, p-nitrophenylphosphatases and leucyl-β naphthylamidases.

(C) To obtain an index of the "sidedness" of these characteristic plasma membrane marker enzymes we have studied isolated cells. We hoped to label the plasma membrane of the isolated cell with an isotopic or fluorescent marker, then to measure the enzymatic activity on the exposed plasma membrane surface, and subsequently to isolate plasma membranes from the labeled cells and reassay the enzymatic activity.

We first approached this problem by the use of milk fat globule membranes. In collaboration with Prof. S. Patton we have prepared mammary gland plasma membranes from milk fractionations. The basic technique was similar to that already outlined. Milk fat globules, in whole milk, are surrounded by mammary gland plasma membrane and the exposed surface is the same as that which is exposed in the milk duct of the gland. Following disruption procedures, both surfaces become exposed. Our observations on the milk fat globule membrane indicated that the 5'-nucleotidase is preferentially located on the external or outer face of the cell membrane, whereas the nucleotide pyrophosphatase may represent the predominant enzyme on the internal or "inside" face of the membrane.

The more substantial difficulties in preparing intact, osmotically-sealed, isolated liver cells have not yet permitted accumulation of more than preliminary data, which could not be discussed at this time with any high degree of confidence.

Significance: The primary significance of our findings rests on the establishment of the nature and quantity of the complex molecules of which the plasma membrane is composed. Although a good deal has been learned about the membrane composition, very little is known about the function of the lipids and proteins in the membrane. At the present time there is no clear indication why such enzymes as the 5'-nucleotidase, nucleotide pyrophosphatase or leucine β-amidase occur in that particular location, but conceivably they may serve as metabolic regulators, perhaps as "door keepers", and possibly even as messengers or inactivators of transmitters in inter-cellular communication.

Proposed Course: Continuation of studies on the composition and function of the plasma membrane along the lines indicated, with a primary objective of obtaining viable cells from guinea pig or rat livers which have an intact surface by all established criteria. Additionally, we hope to develop labeling (or marker) techniques for cell surfaces more adequate than those currently reported. Investigations on the composition of the plasma membrane and on the function of the building blocks which are isolated from such preparations will be continued.

Publications:

1. Lippel, K., Robinson, J., and Trams, E. G.: Intracellular distribution of palmitoyl-CoA synthetase in rat liver. Biochim. Biophys. Acta 206: 173-177, 1970.

2. Robinson, J. S., and Trams, E. G.: Nucleotide pyrophosphatase activity of rat liver plasma membranes. Biochim. Biophys. Acta 219: 93-103, 1970.

3. Franklin, J. E., and Trams, E. G.: Metabolism of coenzyme A and related nucleotides by liver plasma membranes. Biochim. Biophys. Acta 230: 105-116, 1971.

4. Trams, E. G., Lauter, C. J., and Franklin, J. E.: Preparation of 3'-^{32}P-coenzyme A. J. Labeled Compounds, in press.

5. Solyom, A., Bradford, R. H., and Furman, R. H.: Apolipoprotein and lipid distribution in canine serum lipoproteins. Biochim. Biophys. Acta 229: 471-483, 1971.

6. Patton, S., and Trams, E. G.: The presence of plasma membrane enzymes on the surface of bovine milk fat globules. FEBS Letters, in press.

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Perinatal Physiology
National Institute of Neurological Diseases and Stroke

Ronald E. Myers, Chief

The Laboratory of Perinatal Physiology has continued its studies of the neurology of social behavior and affect. The major emphasis, thus far, has been the identification of cortical mechanisms underlying these functions where a major role has been found for the prefrontal and anterior temporal cortex. Some functions require a conjoint activity of these two zones such that identification of deficits require ablation of both zones whereas other functions are affected by removals of either the prefrontal or the anterior temporal cortex. The role of the prefrontal region seems the greater.

Ablations confined to one or the other of these sectors result in major alterations in social behavior. Prefrontal or anterior temporal lesions, for example, result in failure of animals to return to their free-ranging social group despite the presence of family members in the group. Afterwards, they lead solitary existences until removal or death. When confined social groups are studied where the animals are physically bound by the walls of an experimental enclosure, lesions of these cortical regions produce major deficits in the social roles played by the operants. They lose rank in the social order; they fail to interact normally within group social life; they show diminished use of face and voice in emotional expressions; and they become outcasts due to their lack of social affinities and unappropriateness of their social signaling. Prefrontal lobe animals, in addition, exhibit hyperactivity in their patterns of ambulation and bodily movement.

When specific manifestations of social behavior, as maternal behavior, are examined, lesions of prefrontal or anterior temporal cortex produce complete losses. The distressed infant who solicits response from the mother gets no reaction. The mother, instead of gathering up and protecting the infant, ignores or may repel the infant by pushing it away or striking it. The infant may eventually succeed in suckling but only because of its own emotional affinities and needs for the mother. Alternatively, it may be protected by other animals in the group. Among types of social and affective behavior examined, only male reproductive behavior appears uninfluenced by lesions in these areas of cortex.

Prefrontal lobotomies have been performed in the human to diminish levels of anxiety or the manifestations of compulsive behavior. It was thought likely that prefrontal lesions in the monkey would likewise lead to major changes in their expressions of anxiety. Thus, a series of cage-reared monkeys which were overburdened with expressions of anxiety at the approach of the experimenter were subjected to lesions of the prefrontal or anterior temporal cortex. These lesions individually failed to alter the degrees or quality of these monkey anxiety expressions. However, when both areas were lesioned together, the animals lost all manifestations of anxiety permanently. Thus, these two sectors of cortex appeared to function equivalently and conjointly with regard to manifestations of anxiety.

Age and sex play an important role in the expression of deficits following these cortical lesions. Animals less than one year old fail to show deficits clearly apparent in adult members. Thus, maturation is required for the development of these behavioral deficits following lesioning.

In a more restricted laboratory setting, the extent to which facial expression and vocalization in the rhesus monkey may represent patterns of affective behavior has been examined. Lesions of areas in the monkey closely homological to the speech areas in the human fail to result in vocalization changes. By contrast, lesions of prefrontal or anterior temporal cortex lead to major loss in the vocal response of rhesus monkeys. Similar results are demonstrable in relation to facial expression. Thus, in the rhesus monkey, facial expression and vocalization are largely controlled by cortical regions related to affective behavior and minimally controlled by cortical regions that are sensory analytic and nmenonic in function.

Additional studies have demonstrated that vocal response in the rhesus monkey cannot be readily brought under operant control. Thus, present evidence suggests that rhesus facial expression and vocalization are largely involuntary and that they are primarily concerned with social or emotional behavior. This contrasts with the human who has, in addition to this involuntary affective use of face and voice, a volitional control for intraspecific communication functions which is regulated by quite separate regions of the cortex.

Several aspects of CNS development have been studied during the past year. The ontogeny of cerebrospinal fluid ionic composition has been defined. The CSF magnesium concentration is regulated at its adult level (twice serum level) already at the earliest gestational age sampled (sixty days). On the other hand, the CSF potassium concentration reaches adult levels (one-half its dialysable serum concentration) only during the first month of childhood. Evidence from the present and earlier studies indicates that the CSF composition is regulated separately at different levels of the nervous systems by the activity of local blood-brain or blood-CSF barrier mechanisms.

Studies on gestational problems affecting brain development and brain integrity have continued. The implications of placental insufficiency to fetal growth, organ development and the continuation of pregnancy have been studied. Surgical removal of the fetus at different gestational ages does not lead to expulsion of the placenta but to the continuation of "afetal" pregnancy. Following fetectomy, the placenta fails to continue its growth but maintains a constant weight until surgically delivered later.

The production of hormones by the placenta in the absence of the fetus also continues. Placental lactogen concentration in the maternal blood remains constant following fetectomy indicating a close relationship between placental mass and the production of placental lactogen.

When the fetal umbilical vessels supplying the second of the two placental discs are ligated, an insufficient state of the placenta is produced. This leads to a decreased diffusion surface for the passage of respiratory gases and nutrients between the maternal and fetal blood streams. When ligation is carried out at 100 days, a significant retardation in fetal body and organ growth occurs as measured at cesarean delivery near term. The organs most afflicted include spleen, liver, and striated muscle, while least affected is the nervous system. Most affected of brain structures is cerebellum, which also is the brain part most undeveloped and most active in cell proliferation during the latter part of pregnancy. These weight changes closely resemble those observed in human fetal growth retardation or fetal dysmaturity. The chemical composition of the organs of growth-retarded monkey newborns indicates that the organ smallness is associated with smaller cell numbers. However, these cells are normal in cytoplasmic and nuclear masses, contrary to earlier reported work using the rat.

The ultrastructural and light microscopic changes induced in the placenta following fetectomy or fetal umbilical vessel ligation have been defined. Despite the loss of fetal circulation, syncytiotrophoblast continues to exhibit an ultrastructure compatible with a continued full functional activity including hormone secretion. The microvilli lining the maternal surface of the syncytiotrophoblast appears hypertrophic as does the syncytiotrophoblast endoplasmic reticulum and ribosomal apparatus. By contrast, the cytotrophoblast, the Hofbauer cells, and the cells of the villous core of the placental villi, including the fetal capillaries, undergo atrophy, disintegration, and disappearance.

Abruptio placentae represents a serious event in human pregnancy frequently leading to fetal death or fetal brain damage. In addition, abruptio placentae is frequently associated with grave complications for the mother. Disseminated intervascular coagulation associated with dramatic changes in blood coagulability may occur. It is of considerable importance to develop an experimental model of this disease process to aid in the clarification of these dramatic changes in fetal status in utero and in the blood coagulability changes in maternal blood. A model astonishingly close to the human disease has been developed in the Laboratory.

The most informative of the methods for the evaluation of the fetal status in utero is that of scalp sampling. By taking a drop of blood from the fetal scalp by a prick through the vaginal introitus during labor, it is possible to define with precision the fetal respiratory gas and acid-base status. These values in turn precisely define the degree of asphyxia a fetus may have sustained. The disadvantages of fetal scalp sampling are that the method is discontinuous and involves a considerable commitment of manpower and initiative on the part of the physician.

An alternative method for fetal monitoring is the continuous registration of the fetal heart rate or ECG. When the fetus has experienced milder asphyxia, no change may be expected in these parameters. However, when the fetus begins to experience more severe asphyxia, i.e., when the fetal pH is below 7.1-7.05 and the pO_2s below 14 mm Hg, the heart rate begins to slow, exhibiting short bursts of bradycardia (Type II dips) following each uterine contraction. This method of monitoring (detection of Type II dips) has the advantage of a continuous registration of the fetal status but the disadvantage of relative crudity indicating poorly the acid-base and respiratory gas status of the fetus.

Studies in the Laboratory reveal a remarkable stability of the fetal vital signs in the presence of progressive asphyxia. Only when the degree of asphyxial compromise of the fetus reaches extreme degrees do stable changes in the fetal vital signs occur. However, the perturbations of the fetal stable state produced by uterine contractions unmask these vital sign changes early and warn of impending damage or death of the fetus.

Changes in vital signs, including slowing of the fetal heart and drops in fetal blood pressure, develop when the hemoglobin saturation of the fetal blood drops below 15-20 percent. Such levels of hemoglobin saturation occur when the fetal pH falls below 7.10-7.05 and when the partial pressures of oxygen in the fetal blood drop below 12-14 mm Hg.

The Laboratory is currently examining whether the occurrence of bursts of fetal bradycardia following contractions (Type II dips) are compatible with intact survival when they have endured for an hour and have been anteceded by a 3-hour period of mild asphyxia. If the brains of such animals show injury, this would indicate that the utilization of fetal heart rate monitoring is unsatisfactory and is not compatible with intactness of the newborn. On the other hand, if despite this degree of asphyxiation the brains of such animals are intact, one can conclude that fetal heart monitoring is an adequate and reliable method of preventing the occurrence of asphyxial brain damage in the fetus during labor. Present results suggest that fetal heart monitoring may be relatively safe.

The effects of adverse influences on the mother have been studied in relation to alterations in fetal states *in utero*. A variety of manipulations, such as excessive fluothane anesthesia, loss of maternal blood, mechanical constriction of maternal aorta, abruption of placenta, and administration of catecholamines, all result in asphyxia of the fetus, fetal brain damage or fetal death. The relationship between catecholamine stimulation and fetal asphyxia suggests that the endogenous release of catecholamines as by psychic or medical stress may under some circumstances result in fetal brain damage.

The effects of acute total and prolonged partial asphyxia differ profoundly both clinically and pathologically. Acute total asphyxia produces a pattern of injury distributed in the brain stem while involvement of the cortex and basal ganglia is late or nonexistent. On the other hand, prolonged partial asphyxia results in damage distributed within hemispheral structures including the cortex, the white matter, and the basal ganglia. It is these latter patterns of pathology which closely mimic the pathology of human perinatal brain damage which include ulegyria, white matter sclerosis, and status marmoratus of the basal ganglia.

When fetuses at different gestational ages are subjected to episodes of acute total asphyxia, essentially the same brain stem pattern of injury obtains regardless of age. However, the less mature the fetus the less the probability of involvement of the more rostral brain stem structures suggesting the operation of a rostrocaudal gradient of vulnerability corresponding to the rostrocaudal gradient of differentiation. Additionally, the earlier in gestation the episodes of total asphyxia are carried out the longer these episodes must be to produce brain damage. These differences in tolerance are very significant with 3- to 4-fold differences exhibited between fetuses of 50 and 150 days gestational age.

A variety of forms of energy deprivation to the brain lead to hypometabolic cerebral states which result in significant changes in brain glycogen content during recovery. Significant increases in glycogen storage develops which is maximal at 12 hours post-insult. Thereafter, the glycogen content recovers toward normal so that by 24-48 hours normal levels are again achieved. Histochemical and electron microscopic studies have indicated that CNS glycogen is largely contained within astrocytes. Again histochemical studies reveal a peak in storage at 10-14 hours following asphyxial or other injury. Only following hypoglycemia is there departure from the changes thus far described. The glycogen content of the brain following hypoglycemia also peaks at 12 hours, but this high glycogen level continues through at least 60 hours.

The significance of the increase in glycogen of the astrocyte following energy deprivation states is not clear. However, current data suggests a relation to the diminished brain metabolism which follows episodes of energy deprivation. Glucose, which normally may be transported by astrocytes from capillary lumen to intracerebral sites of utilization, may pile up. With glucose pile-up there may develop an increased activity of glycogen-forming enzymes and an associated overproduction of glycogen locally in astrocyte. In support of this interpretation is the fact that glycogen also piles up following a similar time course during deep barbiturate anesthesia and hypothermia.

Twelve to fourteen minutes of total asphyxia result in a doubling of the brain glycogen concentration in the term fetus after 12 hours of recovery. All periods of total asphyxiation between 9 and 30 minutes lead to such build-ups of glycogen in the brain during recovery. Periods of asphyxia less than 9 minutes result in considerably lesser pile-ups of glycogen. However, all lengths of asphyxia (even as short as 3 minutes) produce some increase in brain glycogen content during recovery. Thus, no clear threshold level of asphyxia can be described that relates to post-insult glycogen pile-up.

Electron microscopic studies have been carried out of the extracellular spaces of the brain both in normal and asphyxiated term fetuses using freeze substitution rather than normal fixation to lessen artifactual changes in the extracellular space. During partial asphyxia a major shift of fluid from the extracellular into the intracellular brain spaces develops. The extracellular spaces shrink to the point of disappearance while the cellular extensions enlarge greatly. These changes in fluid distribution between the extracellular and intracellular spaces may occur before gross brain swelling is apparent.

The brains of term monkey fetuses subjected to partial asphyxia have been analyzed for water and electrolyte content. The gray matter of cortex and basal ganglia tend to gain water and lose sodium and potassium. The cerebellar cortex exhibits the greatest, the white matter the least change in water and electrolyte. These alterations accompany and constitute brain swelling.

Prolonged partial asphyxia of the term monkey fetus in utero leads to major changes in regional cerebral perfusion. In extreme cases a total loss of cerebral perfusion may occur. Thus far, no clear correlation has been found between the patterning of changes in cerebral perfusion and the distribution of pathology observed following partial asphyxia.

Studies on the clinical and pathological consequences of hypoglycemia show the rhesus monkey adult can sustain up to 2-3 hours of severe hypoglycemia without injury. During hypoglycemia with glucose levels between 10-20 mg %, the animals exhibit minimal changes in acid-base and respiratory gas status. Following longer episodes, the animals develop severe neurological changes, including severe spastic quadriparesis, blindness, deafness, and dementia. Pathologically, lesions appear in the cerebral cortex, the basal ganglia, and the hippocampus. The convolutions are atrophic, the white matter scanty and the ventricles enlarged. The entire nervous system is infiltrated by wandering cells presumably originating in the circulating blood.

Cyanide infusions lead to striking changes in cardiovascular performance but with lesser changes in acid-base or respiratory gas status except for hyperoxygenation of venous blood. The animals tend either to survive unharmed or to succumb. The limited number of animals surviving cyanide infusions exhibit paracentral cerebral necrosis, a pattern of injury which characterizes many other hypometabolic brain states.

A postmortem model of brain swelling is under examination. Sudden death leads to increased imbibition of fluid and loss of electrolytes from the brain incubated in body temperature saline as compared to prolonged death in severe hypotension. These alterations in the experimental brains closely simulate those found in the human brain following sudden or slow death.

Following circulatory arrest and reperfusion with carbon-black solutions at arterial pressure levels, patterns of failure to reflow correspond in their distribution to the predilection areas for damage observed in a variety of models including hypoxia, cyanide poisoning, perinatal asphyxia, ets. It is believed that no-reflow of blood following circulatory stasis may play an important role in defining the distribution of injury in these models of brain damage.

Serial No. NDS(I) 66-LPP 1386
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental placental insufficiency in the rhesus monkey

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators:	Donald Cheek, M.D.	Prof. Dr. M. Panigel
	Donald Hill, M.D., F.R.C.P.	Henry Friesen, M.D.
	A. B. Holt, A.A.I.M.L.T.	C. Belanger, M.D.
	R. E. Scott, B.S.	B. Shome, M.D.
	E. D. Mellits, B.E.S., Sc.D.	

Cooperating Units: Unité de Physiologie Placentaire, Faculté des Sciences and INSERM, Hopital St. Antoine, Paris, France, Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland, and Protein and Polypeptide Laboratory, Royal Victoria Hospital, Montreal, Canada

Man Years:

Total:	0.8
Professional:	0.1
Other:	0.7

Project Description:

Objectives: 1) The effects of ligation of the fetal placental vessels upon placental morphology. 2) The relationship between placental insufficiency and fetal dysmaturity. 3) The relation between placental insufficiency and brain damage.

Methods Employed: Monkeys at different gestational ages are subjected to hysterotomy. The fetal vessels bridging the space between the primary and the secondary placental cakes are identified and doubly ligated so that the secondary placenta is completely devascularized. Incisions are closed and the fetus brought to term. On cesarean section at term, the gross and microscopic placental morphology is described. The infant is weighed at birth and its various organs weighed with precision after dissection. Weighed samples of the various internal organs, including muscle and bone, are sent to the Department of Pediatrics, Johns Hopkins Hospital, for chemical analysis. The placenta is sent for morphological and histopathological description.

Major Findings: 1) Placental vessel ligation results in considerable alteration in the gross and microscopic morphology of the secondary placenta. These changes have been described in a paper submitted for publication. 2) Dysmaturity of the babies was seen in many cases. The greatest "small-for-dates" observed exhibited a birth weight of only 55% of average for the newborn rhesus monkey. Other animals with lesser degrees of pathological change of the placenta showed lesser degrees of gross retardation. This study is still under progress and more precise quantitative data will be in the offing. 3) Only one animal of the series so far has exhibited neurological abnormalities which consisted of extensor hypertonus and disordered use of the hind extremities with some clumsiness in the hands. This animal, despite six month's survival, failed to develop normal gait. On examination of the brain, there was evidence for astrocytic scarring in the white matter in the predilection zones for perivenular hemorrhage and periventricular leukomalacia which included the white matter in the parasagittal region. In addition, the subependymal astrocytes exhibited a curious hypertrophy almost to the extent of producing a mass lesion in both occipital lobes with less striking such abnormalities throughout the third and lateral ventricles and in the aqueduct of sylvius. This animal failed to exhibit pathological changes in the predilection zones for damage in animals subjected to acute asphyxia.

Significance: The findings relate to the problems of fetal dysmaturity, to placental pathology in relation to alteration of fetal circulation and to brain damage in the perinatal period.

Proposed Course of Project: Data is being analyzed and further manuscripts are in preparation.

Publications:

Myers, R. E., Hill, D., Cheek, D. B., Holt, A. B., Scott, R. E., and Mellits, E. D.: Fetal growth retardation produced by experimental placental insufficiency in the rhesus monkey. I. Body weight, organ size. Biol. Neonate. In press.

Panigel, M., and Myers, R. E.: Histological and ultrastructural changes in monkey placenta following interruption of fetal placental circulation by fetectomy or umbilical vessel ligation. Acta Anat. In press.

Panigel, M., and Myers, R. E.: Embryologie Expérimental.--L'effet de la foetectomie et celui de la ligature des vaisseaux foetaux interplacentaires sur l'ultrastructure des villosités placentaires chez Macaca mulatta. C. R. Acad. Sc. Paris 272: 315-318, 1971.

Serial No. NDS(I) 66-LPP 1387
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental placental abruption in the rhesus monkey and its relation to brain damage

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: David Aronson, M.D.
Prof. Dr. Maurice Panigel

Cooperating Units: Laboratory of Blood and Blood Products, DBS
Hopital St. Antoine, Paris, France

Man Years:

Total:	0.8
Professional:	0.1
Other:	0.7

Project Description:

Objectives: To develop a model of abruptio placenta in experimental animals and to study the resultant brain pathology.

Methods Employed: Monkeys at various gestational ages are subjected to hysterotomy. Abruption of varying proportions of the placenta is produced by finger dissection. Incisions are closed and the animal is brought to term. At term, placental specimens are carefully studied for morphological changes and the fetus evaluated clinically and pathologically for brain damage.

Major Findings: In the exploratory studies thus far completed, abruptions were incompatible with fetal survival in utero because of the development of thromboembolic events in the maternal uterine circulation leading to Couvelaire uterus.

Significance: The demonstration of thromboembolic disease following abruption of the placenta may well explain the occurrence of sudden unexplained death in the patient with a complicated parturition.

Proposed Course of Project: Further studies are necessary for the clarification of the nature of the thromboembolic process observed after abruption.

Publications: None

Serial No. NDS(I) 66-LPP 1388
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Perinatal asphyxia in the monkey and its CNS consequences

Previous Serial Number: Same and incorporating Serial No. NDS(I) 69-LPP 1703, Serial No. NDS(I) 69-LPP 1706, Serial No. NDS(I) 70-LPP 1813, and Serial No. NDS(I) 70-LPP 1828

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators:

Martin Reivich, M.D.	Harvy Shapiro, M.D.
Jacques F. Roux, M.D.	T. Yoshioka, M.D.
Alfred W. Brann, M.D.	Stanley James, M.D.
K. Adamsons, M.D., Ph.D.	E. Mueller-Heubach, M.D.
R. G. Hibbs, Ph.D.	D. E. Phillips, Ph.D.
James A. Miller, Ph.D.	Faith Miller, Ph.D.

Cooperating Units: Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania; Cleveland Metropolitan General Hospital, Cleveland, Ohio; University of Washington, Seattle, Washington; University of Mississippi Medical Center, Jackson, Mississippi; Mt. Sinai School of Medicine, New York; College of Physicians and Surgeons, New York; and Tulane University School of Medicine, Vermillion, S. D.

Man Years:

Total:	0.8
Professional:	0.1
Other:	0.7

Project Description:

Objectives: To determine the patterns of clinical alteration and neuropathological change produced by asphyxiation at the time of birth. To attempt to correlate the patterns of neuropathological change produced with the functional changes occurring in the fetus during asphyxiation.

Methods Employed: Monkey fetuses are delivered by cesarean section at term and subjected to asphyxiation prior to first gasp by the placement of a rubber sac over the head. The lengths of asphyxiation are of predetermined length or are determined by the degree of functional deterioration produced in the fetus. An effort was made to produce the maximum degree of damage compatible with survival. Studies are carried out on the cardiovascular, blood chemical (acid base status), blood gases, pH, lactate, etc., and respiratory changes of the fetus during and after asphyxiation. Resuscitation is carried out using positive pressure oxygen ventilation.

Major Findings: With the varying lengths of asphyxiation there were varying degrees of brain damage clinically and neuropathologically apparent. The most common clinical alteration was sensory loss in the extremities and over the head of varying degrees, alterations in sucking and swallowing, alterations in the voice, and varying degrees of ataxia and clumsiness. In some instances atrophic or traumatic sores occurred over the face and head. With more severe damage, the animals exhibited changes in postural tone and changes in use of extremities with the most severe instances showing almost total loss of use of all four extremities and severe degrees of flexor hypertonus in all four extremities. Pathologically, destructive changes were found in nuclei of predilection in the brain stem and in posterior ventral and lateral ventral nuclei of the thalamus and infrequently in putamen.

In the animals showing more severe involvement, there was extension of damage to increasingly larger amounts of tegmentum of the mid brain, pons and medulla, with involvement also of trigeminal and facial motor nuclei, involvement of the intermediate and ventral portions of the gray matter of the spinal cord, particularly in the lumbar region, and also extension of the destructive process in the thalamus to include progressively larger portions until this structure was literally destroyed except for small nuclear masses medially located. In general, the type and extent of neuropathological alteration could be correlated with parameters of functional alteration in the status of the fetus occurring during the asphyxial process. For example, hemorrhagic changes within the distribution pattern occurred only in instances of profound and prolonged drop in blood pressure with restitution of cardiovascular system status and survival for a period of time. Less commonly, other patterns of neuropathological change were seen such as laminar necrosis, focal cortical atrophy with ulegyria, etc. These changes were seen respectively under circumstances of severe fetal acidosis and of elevated venous pressure in relation to the asphyxial process.

Significance: The intent is to reproduce the patterns of neuropathological change seen in cases of human cerebral palsy and mental retardation. To a good extent, the study has been fruitful in this regard. More important still is the effort to determine the critical parameters involved in the functional deterioration of the fetus during asphyxial insult and to relate these changes to the patterns of neuropathological change produced. Finally, efforts are being made to determine techniques through which parameters of functional deterioration of the fetus may be corrected during the asphyxial insult.

Proposed Course of Project: We hope to extend the number of physiological and blood chemical changes which are measured in relation to the asphyxial process. Efforts will be made to artificially produce alterations in functional status of the various factors determined to be important in order to more clearly define the pathogenesis of brain damage.

Publications:

Roux, J. F., Yoshioka, T., and Myers, R. E.: Conversion of palmitate to respiratory carbon dioxide by foetal tissue of man and monkey. Nature 227: 963-964, 1970.

Myers, R. E.: Models of asphyxial brain damage in the newborn monkey. In Bailey, P., and Fiol, R. E. (Eds.): Second Pan American Congress of Neurology. San Juan, Puerto Rico, Impreso por Los Talleres de Artes Graficos del Departamento de Instruccion, 1970, pp. 214-219.

Myers, R. E.: Closing remarks. Proceedings of the Symposium on Fetal Evaluation During Pregnancy and Labor: Experimental and Clinical Aspects. Milan, Italy, 1971. In press.

Myers, R. E.: Conditions leading to perinatal brain damage in the non-human primate. Proceedings of the Symposium on Fetal Evaluation During Pregnancy and Labor: Experimental and Clinical Aspects. Milan, Italy, 1971. In press.

Myers, R. E.: Two distinct models of perinatal brain damage in the rhesus monkey and their conditions of occurrence. Amer. J. Obstet. Gynec. In press.

Myers, R. E.: Experimental perinatal brain damage in the rhesus monkey. Devel. Med. Child Neurol. In press.

Serial No. NDS(I) 66-LPP 1403
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A study of the prefrontal cortex and related structures

Previous Serial Number: Same

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: R. E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: To investigate functional relations of the prefrontal lobe and related structures in the monkey by behavioral techniques, attempting to develop the functional boundaries of the macaque brain as contrasted to the anatomical ones.

Methods Employed: Nine monkeys were preoperatively trained on delayed response and go-no-go type visual color discrimination tasks. On completion of training, three monkeys underwent bilateral ablation of the anterior one-third of the temporal neocortex, three of the dorsomedial thalamus nuclei, and three served as controls. Two weeks after surgery, the animals were retrained on the two tasks.

On completion of testing, they were further trained on simultaneous object color discrimination, pattern discrimination, pattern discrimination reversals, extinction tests, and position habit reversals.

Major Findings: Immediately after surgery, the temporal animals appeared tamer than preoperatively. Their appetites were lower, two actually losing some weight. These effects disappeared almost completely with time.

Postoperative performance on delayed response showed that neither the temporal nor thalamic removals had any effect. However, on the go-no-go task, rather marked impairments in performance were shown by the temporal monkeys, whereas thalamic monkeys did not differ from the controls.

The temporal animals took more trials to reach criterion and made more errors than the thalamic or the normal animals in the postoperative learning of object color discrimination and pattern discrimination tasks. No differences were found between the latter two groups. Performance on pattern discrimination reversals revealed impairment by the temporal but not by the thalamic animals.

The results of extinction tests revealed no differences among the three groups: but, in position habit reversals, the temporals were again inferior to the other two groups who failed to show differences among themselves.

Significance: The impairments of performance exhibited by the temporal monkeys may be interpreted in part as being due to an impaired ability to inhibit already established response tendencies.

When interpreted in this way, the deficit resembles the deficit described for lesions of orbitofrontal cortex. However, the lack of differences among the groups in extinction tests seems to question this interpretation. Further study is needed to elucidate these questions.

Proposed Course of the Project: Results obtained thus far require publication. Further studies will more closely compare prefrontal with temporal lesions.

Publications: None

Serial No. NDB(I) 66-LPP 1404
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of brain lesions on conditioned vocalization in monkeys

Previous Serial Number: Same

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: R. E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: To investigate brain mechanisms underlying conditional vocalization response in monkeys. Present efforts are directed to a direct comparison of rates of learning of vocalization and of bar-press behavior. Six monkeys were divided into three groups of two each. One group was trained to bar-press when a signal is present but to suppress bar-pressing when the signal is absent. A second group was trained in a similar way but the response required was vocalization. A third group was trained to turn the head-up upon the signal and to suppress this irrelevant skeletal response in the absence of the signal.

After reaching a criterion, the signal conditions were reversed, i.e., the monkey who was trained to bar-press upon a signal was now trained to bar-press when the signal was absent.

Major Findings: Bar-press learning was the quickest of the three response types to be acquired. The head-up response was intermediate. No monkeys succeeded in utilizing vocalization responses to obtain rewards.

The effects of cerebral lesions on spontaneous vocalization was then studied. Lesions of prefrontal and anterior temporal cortex resulted in severe loss of rates of vocalization while cingulate lesions had little effects.

Significance: Neurology of vocalization as a subject of scientific investigation is of importance in relation to speech in man. The unexpected major finding in this project was the extreme difficulty in conditioning vocalization. It appears that vocalization is much less under the control of the mechanism supporting instrumental conditioning. Central mechanisms regulating social behavior also affect vocal behavior.

Proposed Course of the Project: Study will be completed as described.

Publications: None

Serial No. NDS(I) 67-LPP 1464
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemistry of the recovering rhesus monkey from various different states of energy deprivation.

Previous Serial Number: Same

Principal Investigator: Americo Rivera, Jr., Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To study the effects of energy deprivation on the glycogen levels of the brain and other tissues of the rhesus newborn and juvenile monkey. The project includes investigations on:

a) The glycogen changes in brain and other tissues of the juvenile monkey recovering from circulatory arrest.

b) The glycogen levels of brain and other tissues of the newborn rhesus monkey recovering from an episode of prolonged partial asphyxia.

c) The effects of barbiturate anesthesia on brain glycogen in the monkey.

d) The glycogen levels in the tissues of recovering acutely asphyxiated newborn monkeys.

e) The glycogen levels of the brain and other tissues of the juvenile recovering from a 6-hour episode of hypoglycemia (< 20 mg % of blood glucose).

Preparations have begun to expand the study of these tissues by analyzing for the glycogen synthetase enzyme systems. Radioactive glycogen (C-14) is being prepared. This tracer will be used to work out the recovery of various isolation methods for glycogen.

Methods Employed: The deprivation of energy to the brain of the various experimental animals has been accomplished by 1) oxygen deprivation (asphyxia, acute or partial), 2) systemic ischemia, 3) prolonged deep anesthesia, and 4) hypoglycemia.

The brain, heart, lung, liver, spleen, kidney, and muscle (adductor) are rapidly excised from the anesthetized animals at various time intervals of the recovery period and frozen in a mixture of isopentane-isohexane cooled to the temperature of liquid nitrogen. The glycogen levels of these tissues were determined.

Major Findings: It was found that 12 hours following the asphyxia or ischemia episode the glycogen level in the brain peaks to approximately twice the control level. At the same time, the hepatic glycogen reaches its minimal levels. This maximum glycogen content of the brain 12 hours after the insult is being used to assay the threshold amount of asphyxia necessary to bring about this response. Using this criterion, it has been found that 9 minutes of asphyxia will produce a maximum cerebral glycogen accumulation after 12 hours.

The study of the other tissues indicates that the liver of the acutely asphyxiated monkey baby accumulates larger quantities of glycogen than does that of the controls. This could be an indication of a metabolic change brought about by the asphyxia. The liver also has a tendency to have very low glycogen levels when the cerebral glycogen level is abnormally high. This temporal relationship appears to be present in several of our models and may indicate a direct or indirect relationship between both organs. The nature of this relationship, if it exists, is not known. The heart, lung, kidney, and muscle recovered rapidly and showed no residual effect as far as their glycogen content is concerned.

Preliminary data on the brain indicates that the glycogen levels are not as sensitive to prolonged partial asphyxia as polysome integrity and protein synthesis.

It has been found that brain glycogen steadily increases to 170% above normal values over the first 48 hours under anesthesia, in spite of the severe restrictions on caloric intake. The increase in brain glycogen due to restricted caloric intake (starvation) is only 32%. The cerebral glycogen increase of 32% of the thoractomy-sham operated control monkey for the ischemia experiments, which have been maintained without caloric intake for three days, shows that the increase in the cerebral glycogen is due to the limited caloric intake of these animals and not due to the major surgery to which they have been subjected. The glycogen buildup in the brain of anesthetized monkeys follows a different pattern than that seen for asphyxia and ischemia.

Unlike acute asphyxia, ischemia, and anesthesia in the monkey, the brain of hypoglycemic monkeys tends to have its glycogen levels increase steadily for 24 hours and level off at 150% above the control levels. At 60 hours the glycogen levels are still very high. The other tissues show no changes in glycogen concentration over the controls.

Significance: The glycogen response by the brain 12 hours after the onset of an asphyctic or ischemic episode is more sensitive than the morphological changes. The carbohydrate changes are reversible.

The livers of the animals also show alteration in their storage patterns during the recovery period. These investigations have pointed out that different forms of energy deprivation result in different changes in the carbohydrate metabolism of the brain. These changes appear reversible. The data also indicate that a relationship may exist between cerebral and hepatic glycogen levels.

Proposed Course of Project: Investigation of the enzymes of the glycogen biosynthesis pathways will be studied, as well as the ATP levels of the various tissues, to ascertain if the mechanisms of the changes can be elucidated.

Publications: None

Serial No. NDS(I) 69-LPP 1478
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neural control of hand strength in the monkey

Previous Serial Number: Same

Principal Investigator: Gilbert A. Preston, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Albert Einstein School of Medicine, New York, New York

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project terminated

Publications: None

Serial No. NDS(I) 68-LPP 1566
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Functions of the anterior corpus callosum of the monkey

Previous Serial Number: Same

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: R. E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: To determine the functions of the anterior portion of the corpus callosum in monkey.

Methods Employed: Monkeys underwent midline section of corpus callosum, anterior commissure, and optic chiasm. Others received midline section of the optic chiasm alone. Following surgery, all monkeys were taught a delayed response, go-no-go visual color discrimination and delayed alternation tasks with one eye occluded and the arm contralateral to the occluded eye restrained. Tests of transfer of training to the opposite eye and arm combination were then carried out.

Major Findings: The forebrain commissure sectioned monkeys showed fair transfer of delayed response and good transfer of delayed alternation. There was failure of transfer of the go-no-go visual discrimination.

Significance: It was anticipated that monkeys with the forebrain commissures sectioned would show lack of transfer of training on the delayed response and the delayed alternation tasks as well as the go-no-go discrimination.

Proposed Course of Project: The present results require a modification of thinking with regard to the neurology of the delayed response and delayed alternation performances. Commissural lesions will be extended to subcortical systems.

Publications: None

Serial No. NDS(I) 68-LPP 1569
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The neuroanatomical basis of reproductive activity in male rhesus monkeys

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Elizabeth A. Missakian, Ph.D.
Luis del Rio

Cooperating Units: None

During FY 1971 this project was incorporated with Serial No. NDS(I) 70-LPP 1825.

Serial No. NDS(I) 69-LPP 1699
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Functions of forebrain commissures in visual learning transfer in the monkey

Previous Serial Number: Same

Principal Investigator: R. E. Myers, M.D., Ph.D.

Other Investigators: Kazuo Hara, Ph.D.

Cooperating Units: Department of Psychology, International Christian University, Mitaka, Tokyo, Japan

Man Years:
Total: 0.5
Professional: 0.15
Other: 0.35

Project Description:

Objectives: To determine the functional role of the corpus callosum and the anterior commissure in interocular transfer of visual learning, the degrees of transfer deficits in relation to the complexity of visual stimuli and the difficulty of discrimination problems (Exp. I), timing of the commissurotomy in relation to the progress of learning (Exp. II), and the degree of early sensory deprivation (Exp. III) were examined.

Methods Employed: Exp. I. Midsagittal sectioning of the optic chiasm, the corpus callosum and the anterior commissure: learning of visual discriminations by one eye, while the other eye is occluded, transfer test after shifting the occluder, reversal learning and subsequent second transfer test with the opposite eye. Five different discriminanda are studied - brightness, color, pattern, form and scene.

Exp. II. General procedure is the same as the Exp. I except that discriminanda utilizing different stimuli are presented in random order. Midsagittal sectioning of the corpus callosum and the anterior commissure is postponed until the subjects complete initial learnings of the third problem.

Exp. III. During various postnatal periods, infant monkeys are subjected to midline sections of the optic chiasm, the anterior commissure and the corpus callosum. At the same time, the eyelids of one eye are sutured closed.

These subjects are raised with monocular vision for twelve months, and the closed eye then reopened. The testing procedure as employed for the Exp. I will be followed.

Major Findings: Exp. I. Monkeys with total section of the forebrain commissures and the optic chiasm exhibited moderate degrees of interocular transfer of brightness, color and scene, while no such transfer in pattern and form was observed. Those stimuli which manifest transfers are available in the natural habitat and thus have become components of social signal, which might be handled in a quantitatively different fashion than neutral signals by the visual mechanisms. Exps. II and III currently being initiated.

Significance: Though the lack of interocular transfer of visual learning after midline section of commissural fibers has been observed by many investigators, many ambiguities on the nature of those deficits still remain. The present study aims to clarify some of the issues which relate to such variables as the difficulty of the discrimination problems and the complexity of the stimuli as well as the degree of sophistication of subjects and the interproblem transfer or generalization of learning tasks. Also, the last experiment would provide critical tests for the time course of development of visual perception and visual learning and of commissural functions in relation to these processes. Findings from these studies have relevance for our understanding of the rehabilitation of human blindness (amblyopia ex anopsia) as well as for the psychological understanding of sensory deprivation and its effects.

Proposed Course of Project: Exp. I completed. Exps. II and III have been initiated according to the plan.

Publications:

Myers, R. E.: The forebrain commissures and their functions. Cerebral Interhemispheric Relations. In press.

Myers, R. E.: The corpus callosum and its functions: Animal experimental work. In Bailey, P., and Fiol, R. E. (Eds.): Second Pan American Congress of Neurology. San Juan, Puerto Rico, Impreso por Los Talleres de Artes Graficos del Departamento de Instruccion, 1970, pp. 234-242.

Serial No. NDS(I) 69-LPP 1703
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effects of rapid correction of acid-base abnormalities on the brain pathology seen following acute total asphyxia

Previous Serial Number: Same

Principal Investigator: Stanley James, M.D.

Other Investigators: A. W. Brann, Jr., M.D.
K. Adamsons, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: College of Physicians and Surgeons of Columbia University, New York, University of Mississippi Medical Center, Jackson, Mississippi, and Mt. Sinai School of Medicine, New York

During FY 1971 this project was incorporated with Serial No. NDS(I) 66-LPP 1388.

Serial No. NDS(I) 69-LPP 1705
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hypoglycemic brain damage in the monkey: An experimental model

Previous Serial Number: Same

Principal Investigator: Kenneth J. Kahn, M.D.

Other Investigators: R. E. Myers, M.D., Ph.D.

Cooperating Units: Psychobiology Laboratory, The John F. Kennedy Center for Research on Education and Human Development, George Peabody College for Teachers, Nashville, Tenn.

Man Years:
Total: 0.4
Professional: 0.05
Other: 0.35

Project Description: Project completed

Publications:

Kahn, K. J., and Myers, R. E.: Insulin-induced hypoglycemia in the non-human primate. I. Clinical consequences. *Spastics International Medical Publications*. In press.

Myers, R. E., and Kahn, K. J.: Insulin-induced hypoglycemia in the non-human primate. II. Long-term neuropathological consequences. *Spastics International Medical Publications*. In press.

Serial No. NDS(I) 69-LPP 1706
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of hypothermia on the neuropathology seen following acute total asphyxia

Previous Serial Number: Same

Principal Investigators: James A. Miller, Ph.D., D.Sc.
Faith Miller, Ph.D.

Other Investigators: A. W. Brann, Jr., M.D.
Stanley James, M.D.
Karlis Adamsons, M.D.
Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Tulane University School of Medicine, New Orleans, Louisiana; University of Mississippi Medical Center, Jackson, Mississippi; College of Physicians and Surgeons, New York, New York; and Mt. Sinai School fo Medicine, New York, New York

During FY 1971 this project was incorporated with Serial No. NDS(I) 66-LPP 1388.

Serial No. NDS(I) 69-LPP 1710
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Changes in the nucleic acid content of adult monkey brain incident to hypoglycemia

Previous Serial Number: Same

Principal Investigator: Robert O. Petersen, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Massachusetts General Hospital, Boston, Massachusetts

Project Description: Project terminated

Publications: None

Serial No. NDS(I) 69-LPP 1711
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Callosal localization of transfer of tactuokinesthetic information in the rhesus monkey

Previous Serial Number: Same

Principal Investigator: Gilbert A. Preston, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Albert Einstein School of Medicine, New York

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project terminated

Publications: None

Serial No. NDS(I) 69-LPP 1715
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of hypoxic hypoxia on the monkey brain

Previous Serial Number: Same

Principal Investigator: Harvey M. Shapiro, M.D.

Other Investigators: R. E. Myers, M.D., Ph.D.
R. Hibbs, Ph.D.
R. Petersen, M.D., Ph.D.

Cooperating Units: Division of Neurosurgery and Department of Neurology, University of Washington, Seattle, Washington, and Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts

Man Years:

Total:	0.8
Professional:	0.1
Other:	0.7

Project Description:

Objectives: The hypoxic hypoxia model is developed for study of brain changes associated with hypoxia in the absence of arterial hypotension or circulatory stasis. This preparation isolates the effect of oxygen deprivation upon brain chemistry, acid-base status, electrical activity, and morphology.

Methods Employed: Under intravenous barbiturate anesthesia, the adult monkey is exposed to a 2.5-3.5% mixture of oxygen in nitrogen administered by a mechanical ventilator which is adjusted to yield a mild hypocapnia.

Cerebrospinal fluid and blood gas tensions and acid-base status are monitored. Small amounts of cardiotonic drugs are administered to permit adjustment of the arterial blood pressure. The jugular venous pressure, electroencephalogram, brain electrical impedance, electrocardiogram, and at times the intracranial pressure are monitored. Animals for optical microscopy survive for a period of three weeks and are then formalin-perfused and their brains examined grossly and histologically. Animals for electronmicroscopy are perfused with glutaraldehyde at different stages of insult and recovery. Luciferene assays for ATP levels are performed on animals sacrificed at various times during hypoxia and recovery.

Major Findings: Hypoxia without vascular hypotension results in an isoelectric EEG in 3-8 minutes in the present experimental procedure utilizing 2.5-3.5% oxygen mixtures. The arterial pO_2 is 18 and the jugular venous pO_2 is 12. Both arterial blood and cerebrospinal fluid develop marked acidosis. There is a significant decrease in CSF bicarbonate. Administration of 100% oxygen after the insult period results in a jugular venous blood hyperoxia as manifested by a pO_2 range of 275-310. The duration of the jugular venous hyperoxia appears to relate to the duration of the hypoxia, when arterial blood pressure is maintained over a mean of 80 mm Hg. Below mean arterial pressures of 65, the degree of hyperoxia is enhanced and the hyperoxic period prolonged. This result indicates a greater effect upon the brain of the standard hypoxic insult at lower arterial perfusion pressures.

The hypoxic insult results in a significant systemic and cerebrospinal fluid acidosis. Animals with the higher range of perfusion pressures have lesions limited to the globus pallidus. Biochemical analysis reveals a tenfold decrease in cortical ATP soon after the onset of 3.5% O_2 administration. Brain electrical impedance increases 20-30% with an onset latency of 2-4 minutes. The impedance returns toward its baseline values rapidly after the administration of 100% oxygen if there has been no hypotension.

Significance: Remarkable degrees of resistance of the brain to profound hypoxia are demonstrated if the blood pressure is maintained above a mean of 65 mm Hg. Prolonged jugular venous hyperoxia is a sign of a severe insult to the brain. The recovery slope of the J.V. hyperoxic phase may have prognostic value with clinical applications.

Proposed Course of Project: Analysis of data and neuropathological changes are in progress.

Publications: None

Serial No. NDS(I) 70-LPP 1810
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Vital signs of the fetus as indicators of the state of fetal oxygenation

Previous Serial Number: Same and incorporating Serial No. NDS(I) 70-LPP 1811

Principal Investigator: Karlis Adamsons, M.D., Ph.D.

Other Investigators: E. Mueller-Heubach, M.D.
Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine and College of Physicians and Surgeons of Columbia University, New York, New York

Man Years:
Total: 0.95
Professional: 0.1
Other: 0.85

Project Description:

Objectives: To determine the value of fetal heart rate and blood pressure in the surveillance of the oxygenation of the fetus during labor and delivery.

Methods Employed: Fetal rhesus monkeys near term are partly delivered by hysterotomy and prepared for recording of heart rate, blood pressure and intermittent blood sampling. The fetus is then returned to the uterine cavity; the uterus and the abdominal wall of the mother are closed while an occluding device is retained around the maternal aorta for a stepwise reduction in intervillous space perfusion. Blood samples of mother and fetus are obtained at intervals for pH, pO_2, pCO_2 and base deficit measurements.

Major Findings: Fetal rhesus monkeys near term are partly delivered by mild, moderate degrees of fetal asphyxia. Only when the oxygen content of fetal arterial blood falls below 25% of normal is there a consistent decrease in fetal heart rate.

Significance: This study has demonstrated that even a continuous recording of fetal heart rate during labor may fail to identify states of oxygen deprivation that might be associated with permanent damage of the CNS, and that analysis of fetal blood is indispensable if such occurrences are to be eliminated.

Proposed Course of Project: The study is nearly completed and data are being analyzed for the preparation of a manuscript.

Publications:

Adamsons, K., Mueller-Heubach, E., and Myers, R. E.: The innocuousness of amniotic fluid infusion in the pregnant rhesus monkey. Amer. J. Obstet. Gynec. 109: 977-984, 1971.

Serial No. NDS(I) 70-LPP 1811
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The relation between fetal ECG and acid base state

Previous Serial Number: Same

Principal Investigator: Eberhard Mueller-Heubach, M.D.

Other Investigators: Karlis Adamsons, M.D., Ph.D.
Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine, New York, New York, and College of Physicians and Surgeons of Columbia University, New York, New York

Project Description:

During FY 1971 this project was incorporated with Serial No. NDS(I) 70-LPP 1810.

Serial No. NDS(I) 70-LPP 1812
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of a reduction of fetal metabolic needs upon the duration of gestation

Previous Serial Number: Same

Principal Investigator: Karlis Adamsons, M.D., Ph.D.

Other Investigators: E. Mueller-Heubach, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine, New York, New York, and College of Physicians and Surgeons, Columbia University, New York, New York

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project terminated

Publications: None

Serial No. NDS(I) 70-LPP 1813
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The role of epinephrine in the resuscitation of the asphyxiated fetus

Previous Serial Number: Same

Principal Investigator: Karlis Adamsons, M.D., Ph.D.

Other Investigators: E. Mueller-Heubach, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine, New York, New York, and College of Physicians and Surgeons of Columbia University, New York, New York

During FY 1971 this project was incorporated with Serial No. NDS(I) 66-LPP 1388.

Serial No. NDS(I) 70-LPP 1814
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of catecholamines upon the perfusion of the intervillous space

Previous Serial Number: Same

Principal Investigator: Karlis Adamsons, M.D., Ph.D.

Other Investigators: E. Mueller-Heubach, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine, New York, New York, and College of Physicians and Surgeons of Columbia University, New York, New York

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project completed.

Publications:

Adamsons, K., Mueller-Heubach, E., and Myers, R. E.: Production of fetal asphyxia in the rhesus monkey by administration of catecholamines to the mother. Amer. J. Obstet. Gynec. 109: 248-262, 1971.

Serial No. NDS(I) 70-LPP 1815
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A model for the _in vivo_ measurement of oxygen consumption of the brain

Previous Serial Number: Same

Principal Investigator: Eberhard Mueller-Heubach, M.D.

Other Investigators: K. Adamsons, M.D., Ph.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: College of Physicians and Surgeons of Columbia University, New York, New York, and Mt. Sinai School of Medicine, New York, New York

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project terminated.

Publications: None

Serial No. NDS(I) 70-LPP 1817
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Placental pathology and anomalies in the rhesus monkey

Previous Serial Number: Same

Principal Investigators: Ronald F. DiGiacomo, V.M.D.
Marion Valerio, D.V.M.
Thomas O. McCann, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D. C., and Presbyterian Medical Center, The Presbyterian Hospital, New York

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To determine and classify the anomalies and abnormalities of the rhesus monkey placenta.

Methods Employed: Over the past 2-3 years, many abnormalities of the rhesus monkey placenta have been noted. These abnormal placentas as well as normal placentas collected at time of hysterotomy have been preserved in 10% formalin for subsequent study.

Major Findings: This project has only recently been initiated as regards thorough examination of the individual placentas, but the following abnormalities have already been noted: circumvallate placenta, placenta previa, and the presence of a single umbilical artery. Various apparent infarctions of the placenta have also been noted.

Significance: This study is being conducted to determine some of the spontaneously occurring abnormalities of the rhesus monkey placenta in order to establish background information for experimental studies being conducted.

Proposed Course of Project: To continue to examine both grossly and microscopically rhesus monkey placentas to firmly establish spontaneously occurring abnormalities in this species.

Publications:

DiGiacomo, R. F., and McCann, T. O.: Gynecologic pathology in the *Macaca mulatta*. *Amer. J. Obstet. Gynec.* 108: 538-542, 1970.

Serial No. NDS(I) 70-LPP 1818
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experiences with a large breeding colony of *Macaca mulatta*

Previous Serial Number: Same

Principal Investigators: Ronald F. DiGiacomo, V.M.D.
Peter W. Shaughnessy, Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Office of Biometry, NINDS

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To describe experiences with a 600-member rhesus female breeding colony encompassing a period of about 13 years.

Methods Employed: In the course of management of a large rhesus breeding colony many normative determinations have been made on the pregnant rhesus monkey. This data has been collected and is now being subjected to statistical analysis. A sampling of the topics to be covered include correlation of placental and birth weight, menstrual cycle length and range, mating and conception experience, study of fetal mortality, amniotic fluid pH during gestation, sedimentation rate during gestation, harem breeding, induced vaginal delivery, gynecologic and obstetric problems, question of seasonality in the laboratory, rhesus prematurity, etc. The amount of data is voluminous; the foregoing list is only a brief summary of some of the areas to be studied.

Major Findings: The initial findings to date concern the prediction of gestational age and birth weight. Utilizing measurements obtained from bimanual rectal palpation of the uterus and height of fundus, graphs have been drawn for the prediction of gestational age in the female from known timed pregnancies. An analysis has also been made of the factors which influence birth weight and three factors were noted to have equal value in this determination: pregnant maternal weight, maternal weight gain, and gestational age.

Significance: This study will provide baseline data or a large sampling from an uncontrolled environment. This information is necessary to plan more controlled experiments in the area of fetal and neonatal physiology.

Proposed Course of Project: All of the data on the various subjects to be examined has now been collected. Most of the data has already been put on the computer and analysis of it is already under way. One manuscript is already in preparation, "Prediction of Gestational Age and Birthweight in the Rhesus Monkey (*Macaca mulatta*)." This segment will take at least several months for completion.

Publications: None

Serial No. NDS(I) 70-LPP 1820
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Protein synthesis by cerebral ribosomes in chronically asphyxiated term fetal monkeys

Previous Serial Number: Same

Principal Investigator: Stanley Holstein, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Duke University Medical Center, Durham, North Carolina

Man Years:

Total:	0.4
Professional:	0.05
Other:	0.35

Project Description: Project terminated

Publications: None

Serial No. NDS(I) 70-LPP 1821
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of local hypoventilation on brain tissue water and electrolyte contents in rhesus monkeys

Previous Serial Number: Same

Principal Investigator: Michael E. Selzer, M.D., Ph.D.

Other Investigators: S. B. Holstein, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Duke University Medical Center, Durham, North Carolina

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: To develop a model of brain edema using local tissue asphyxia, and to determine its effects on the distribution of brain water and electrolytes.

Methods Employed: With the common carotid artery clamped, blood from the inferior vena cava is delivered at physiological pressures into the internal carotid of a 3.5-6 kg rhesus monkey via a peristaltic pump, and a catheter in the external carotid threaded retrograde to the carotid bifurcation, unilaterally. The animal is hypoventilated until venous pO_2 is 20 mm Hg or less. By this means, severely desaturated blood can be delivered to one hemisphere, while the rest of the animal receives enough oxygen to prevent cardiovascular collapse. After a one-hour insult, normal respiration and carotid circulation are reestablished, and the animal allowed to recover for varying lengths of time. After recovery, water and electrolyte contents are measured in portions of cerebral cortex, caudate nucleus, cerebral white matter and cerebellar cortex. The cross perfused hemisphere is compared to the contralateral hemisphere, to sham-operated control hemispheres perfused with normally oxygenated arterial blood, and to unoperated controls.

In addition to these experiments, brain tissues from term fetuses undergoing prolonged partial asphyxia by maternal abdominal aorta compression are also sampled for water and electrolytes.

Major Findings: After a three-hour recovery period, the cross circulated hemispheres often show gross evidence of acute parietal and frontal cortical and basal ganglionic necrosis. Water, sodium and chloride contents are increased in gray matter. Potassium is unchanged. White matter is less affected than gray. The contralateral hemisphere is unaffected. Other durations of recovery have been tested, but not yet in a sufficient number of cases to allow generalizations to be made.

In the term fetus prolonged partial asphyxia model, preliminary data suggests that after three hours of fetal arterial pH below 7.1 there is an increase in water and a decrease in sodium and potassium content in gray matter.

Significance: The cross circulation model for asphyctic brain damage is an important step in the study of local hypoxia on the brain. The unilateral nature of the damage seen in our experiments shows that it is possible to control perfusion pressures so that there is minimal cross perfusion through the circle of Willis. Previous models of cerebral anoxia have employed either ligation of cerebral vessels or total body asphyxia. Both of these methods introduce variables which are impossible to control for after only three hours of recovery; those brains which do not show acute necrosis show little in the way of water and electrolyte changes. Thus, it could be argued that our brain edema is merely a blood exudation secondary to capillary destruction. However, in a few 24-hour survivors, we have seen increased water, sodium, and chloride contents in areas of gray matter which show no gross signs of necrosis. The fact that the term fetuses exposed to three hours of prolonged partial asphyxia tend to lose sodium and potassium and gain water without signs of acute necrosis suggest that the brain edema of our asphyctic models will prove to be derived from mechanisms other than the structural breakdown of blood brain barrier.

Proposed Course of Project: The analysis of brain water and electrolytes in the term fetal prolonged partial asphyxia model is being written up for publication. A similar series using 130-day-old fetuses is just beginning.

The cross circulation experiments will be continued, hopefully, with the following new features: a) a decrease in the duration of insult, b) an analysis of brains at longer recovery periods to try to elicit a metabolic, non-necrotic brain edema in the presence of an intact blood brain barrier, and c) the preparation of long-term survivors for histological identification of patterns of brain damage produced during the insult.

Publications: None

Serial No. NDS(I) 70-LPP 1823
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Brain stem mechanisms underlying visually guided behavior

Previous Serial Number: Same

Principal Investigator: Robert Thompson, Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Louisiana State University, Baton Rouge, Louisiana

Project Description: Project completed.

Publications:

Thompson, R., and Myers, R. E.: Brain stem mechanisms underlying visually guided responses in the rhesus monkey. J. Comp. Physiol. Psychol. 74: 479-512, 1971.

Serial No. NDS(I) 70-LPP 1824
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neurology of vocalization in rhesus monkeys

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: S. Yamaguchi, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	0.2
Other:	0.9

Project Description:

Objectives: To define the loci in the CNS responsible for vocalization in the rhesus monkeys.

Methods Employed: Monkeys were selected for high rates of spontaneous vocalization in the experimental situation. The stability of daily vocalizations of each was examined for twenty experimental sessions. The animals were then subjected to one of the four brain operations including: 1) ablation of cortical areas corresponding to human speech areas, 2) ablation of the anterior temporal neocortex, 3) removal of the prefrontal neocortex, and 4) removal of cingulate cortex. After operation, twenty more experimental sessions were run, and the vocalization rates were compared with the preoperative levels. In some cases, the monkeys received second and third operations.

Major Findings: Prior to operation, the rates of spontaneous vocalization of the selected monkeys were generally stable from day to day. The ablation of the cortical areas corresponding to human speech areas tend to have little or no effect on the vocalization rates. The cingulate lesions had slight effects on vocalization. The ablation of the anterior temporal neocortex caused major loss of vocalization with partial later recovery. The removal of prefrontal lobe resulted in permanent and almost total loss of vocalization.

Significance: Little is known about the neural mechanism involved in vocalization in sub-human species. The present effort attempts to clarify and define the neurological basis of vocal behavior in a sub-human primate.

Proposed Course of Project: The next step in the analysis involves study of subcortical mechanisms.

Publications: None

Serial No. NDS(I) 70-LPP 1825
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neurology of affective behavior

Previous Serial Number: Same and incorporating Serial No. NDS(I) 68-LPP 1569

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Shun-ichi Yamaguchi, Ph.D.
Kazuo Hara, Ph.D.

Cooperating Units: Department of Psychology, International Christian University, Mitaka, Tokyo, Japan

Man Years:
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: To investigate the roles of forebrain structures on the manisfestation of emotional states, namely threat and attack.

Methods Employed: Opossum, mongoose, and rhesus monkeys were employed as experimental subjects. Daily observation and recording of their behavioral characteristics toward a variety of stimuli; bilateral removal of one of the following forebrain structures: neocortex, pyriform cortex, n. amygdala, septum, n. hippocampus, n. caudate, area innominata and olfactory bulbs. Postoperative observation: Histological examination of the lesions and the thalamic retrograde degenerations.

Major Findings: In monkeys, bilateral anterior temporal neocortex removal temporarily abolished such emotional reactions as grimacing, agitation, and vocalization. Bilateral prefrontal lobectomy also reduced the same emotional reactions mildly. However, when both of these lesions are combined, the abolishment of the emotional reactions was almost total and long lasting.

Significance: Facial expression and posture, as well as vocalization, manifest emotional states of the organism and serve as significant media for communication. The present study aims to analyze roles of forebrain structures on these expressive behaviors with special efforts offered in two ways:

1) long-term observation through both pre- and post-operative periods, and
2) a wide range of lesions involving diverse structures with forebrain.

Results obtained strongly support a scheme of stratified organization of emotional expressions consisting of neocortical inhibitory, limbic excitatory and subcortical reflectory responses. Applications for clinical diagnostic methods were under development.

Proposed Course of Project: To complete observations.

Publications: None.

Serial No. NDS(I) 70-LPP 1826
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Relationship of age to brain water and electrolyte contents in rhesus monkeys

Previous Serial Number: Same

Principal Investigator: Michael E. Selzer, M.D., Ph.D.

Other Investigators: S. B. Holstein, M.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Duke University Medical Center, Durham, North Carolina

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: To determine how the distribution of water and electrolytes (Na^+, K^+, and Cl^-) in the primate brain changes with morphologic maturation.

Methods Employed: The brains of rhesus monkeys, ranging in age from 40-day fetuses to adults, are removed under barbiturate anesthesia. The water and electrolyte contents of cerebral cortex, cerebral white matter, caudate nucleus, cerebellar cortex, and cerebellar peduncles are determined by standard methods.

Major Findings: All tissues lose water as the monkeys age. However, during the period between the weaning and onset of puberty (second year of life) white matter water loss is still fairly rapid, whereas the water content of gray matter has nearly stabilized, with a very gradual loss of water continuing into adulthood. Sodium and chloride peak briefly during second trimester and then decrease in concentration (when expressed as meq per L of tissue water), especially during the last trimester of gestation and first year postnatally, while potassium concentration rises sharply during this time in all brain tissues studied.

Significance: These studies were initially undertaken when it was noted that the water contents of brains of control animals, in a study of experimentally induced brain edema, had disturbingly wide variations. In primate research young monkeys are often preferred because they are smaller, easier to handle and physiologically resilient. These studies show the importance of taking maturational changes into account when dealing with brain water and electrolytes in non-adult animals.

The relatively rapid decrease of water contents of white matter during a time when gray matter hydration has become relatively stabilized is probably a reflection of continuing myelinization approximately up to the time of puberty.

The observed changes in electrolyte contents in brain total water are significant for their relationship to the questions of cellular functional maturation and the developmental changes in extracellular space. Potassium concentration in cerebrospinal fluid of primates is known to decrease during maturation. Therefore, the increase in brain water potassium levels must reflect an increased intracellular potassium. This supports the thesis that extracellular space decreases during maturation. Calculations based on estimates of extracellular space changes from the literature suggest that the actual intracellular concentration of potassium remains fairly constant.

Proposed Course of Project: Data is being analyzed by computer.

Publications: None

Serial No. NDS(I) 70-LPP 1827
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A fine structural study of gliogenesis in the normal fetal rhesus monkey

Previous Serial Number: Same

Principal Investigator: Dwight E. Phillips, Ph.D.

Other Investigators: None

Cooperating Units: Department of Anatomy, University of South Dakota, Vermillion, South Dakota

Project Description: Project terminated.

Publications:

Phillips, D. E.: An electron microscopic study of glial cell types in the fetal monkey spinal cord. Doctoral Dissertation. Department of Anatomy, Tulane University School of Medicine.

Serial No. NDS(I) 70-LPP 1828
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An electron microscopic (EM) study of cytological changes in cerebral cortex and basal ganglia following prolonged partial asphyxia in the term fetal rhesus monkey

Previous Serial Number: Same

Principal Investigator: R. G. Hibbs, Ph.D.

Other Investigators: D. E. Phillips, Ph.D.
R. E. Myers, M.D., Ph.D.

Cooperating Units: Tulane University, New Orleans, Louisiana, and University of South Dakota, Vermillion, South Dakota

During FY 1971 this project was incorporated with Serial No. NDS(I) 66-LPP 1388.

Serial No. NDS(I) 71-LPP 1899
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical and neuropathological features of experimental carbon monoxide intoxication in the primate

Previous Serial Number: None

Principal Investigator: Myron D. Ginsberg, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Objectives: To characterize the clinical features of acute sub-lethal doses of carbon monoxide in the rhesus monkey; to develop, if possible, a model of cerebral edema and/or selective white matter necrosis using this toxin; to attempt to distinguish the primary neural effects of this toxin from cerebral changes which are secondary to systemic circulatory alterations.

Methods Employed: Timed exposure, via tracheostomy, of anesthetized monkeys to 1 and 3 parts per million of inhaled carbon monoxide gas. Blood pH, pCO_2, pO_2, O_2Hgb, CO-Hgb, and cardiac, electroencephalographic, and clinical variables are followed during the acute interval. Immediate and delayed neurological sequelae are sought for in the surviving animals. Neuropathologic examination is performed on those animals dying acutely and those who survive for periods of days to weeks.

Major Findings: Not yet available.

Significance: The study will elucidate the pathophysiology of carbon monoxide intoxication.

Proposed Course of Project: The bulk of the study will be carried out during the forthcoming year.

Publications: None

Serial No. NDS(I) 71-LPP 1900
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effects of total cerebral circulatory arrest on the patency of the cerebral microvasculature in juvenile rhesus monkeys

Previous Serial Number: None

Principal Investigator: Myron D. Ginsberg, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.9
Other:	0

Project Description:

Objectives: To determine whether the capillary "no-reflow" phenomenon is present in the monkey following total cerebral ischemia of various durations; if present, to document its temporal features and some factors influencing its occurrence.

Methods Employed: Animals are anesthetized; the chest is quickly opened and all the major vessels emanating from or entering the heart clamped. Following ischemia of varying durations, the brain is perfused either with a colloidal carbon suspension or with Ringer's solution. Following formalin fixation, the brains are sectioned (and, in the case of the Ringer's perfused animals, stained for hemoglobin) and examined for capillary perfusion patency.

Major Findings: Clearly demonstrable perfusion defects were noted in the sectioned brains of those animals undergoing 10-60 minutes of ischemia. At 10 minutes the defects were small and limited to symmetrical foci in basal ganglia and paracentral cortex. With increasing ischemia the zones of nonperfusion became more extensive, involving often symmetrical portions of pre- and post-central cortex, middle frontal gyrus, and parieto-occipital junction, as well as thalamus and brain stem. The pattern of cortical nonperfusion tended to resemble in topography the lesion caused by prolonged partial asphyxia in the newborn rhesus. Experiments are still in progress.

Significance: The results, when the study is completed, will provide evidence hitherto lacking as to the temporal features of microvascular change during ischemia. The data will be compared with clinical and neuropathological data already obtained in this species so as to arrive at a more comprehensive picture of the pathophysiology of cerebral anoxia.

Proposed Course of the Project: Results will be verified using a second series of animals.

Publications: None

Serial No. NDS(I) 71-LPP 1901
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neuropathological effects of sodium cyanide in rhesus monkeys

Previous Serial Number: None

Principal Investigator: Michael J. Labance, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: To determine the neuropathological effects of sodium cyanide, its dose dependence, reversibility, and areas of primary effect in the rhesus monkey.

Methods Employed: Monkeys are anesthetized with 35 mg/kg pentobarbitol. A tracheotomy is performed. A femoral artery and both femoral veins are catheterized. Arterial pressure, venous pressure, an EEG and and EKG are monitored. Control blood gases are obtained and subsequently followed throughout the experiment. An intravenous infusion of one mg sodium cyanide per cc of physiological saline is started. Initial experiments utilized multiple single doses. More recent experiments incorporate a constant infusion which is adjusted to a maximal rate which still permits adequate circulatory perfusion. After two hours of intravenous administration, the animal is sacrificed and perfused with saline followed by a thorough perfusion with formalin. The brain is then removed and preserved in formalin.

Major Findings: The multiple single dose regimen of sodium cyanide administration produced marked effects, all dosage dependent, on respiratory and circulatory function. The initial effect of a maximum tolerable dose was hyperpnea and mild blood pressure elevation. A subsequent period of respiratory depression and apnea would characteristically ensue with a corresponding marked hypotensive episode. A slow return to control conditions would then follow. In some instances, bilateral, tonic, clonic seizure activity would occur.

During the continuous infusion experiments hyperpnea was prolonged and a moderate rate of fall of blood pressure was achieved. After a significant total dose of sodium cyanide was given, respiratory activity would wane and assisted respiration was necessary.

No gross edema was apparent at the time of removal of the brains.

Significance: Sodium cyanide reacts with iron in the ferric (trivalent) state and therefore binds to the iron of cytochrome oxidase to form a cytochrome oxidase-CN complex which effectively blocks cellular respiration producing a cytotoxic hypoxia. This model fits into this Laboratory's goal of investigating various methods of energy deprivation in the central nervous system.

Proposed Course of Project: In subsequent experiments, monkeys will be permitted to survive so that evaluation of evolved neurological deficits may be made.

Publications: None.

Serial No. NDS(I) 71-LPP 1902
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neuropathological, physiological and metabolic effects of systemic hypotension on adult monkey brain

Previous Serial Number: None

Principal Investigator: Dennis J. Selkoe, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

1) To develop a satisfactory model of systemic hypotension and the shock syndrome in rhesus monkeys.

2) To investigate the effects of systemic hypotension on:

   a) Characteristic neuropathological patterns in animals surviving varying degrees of hypotension.

   b) Rate and extent of postmortem cerebral changes, particularly brain swelling and distribution of brain water and electrolytes.

   c) Cardiovascular, electroencephalographic and cerebral metabolic parameters.

Methods Employed: Anesthetized juvenile monkeys undergo tracheostomy and catheterization of several vessels. A number of physiologic parameters (B. P., jugular venous pressure, ECG, EEG, temperature, etc.) are continuously recorded. In the experimental animals, systemic hypotension is produced by controlled hemorrhage for varying intervals. After the hypotensive period, the animal is either sacrificed or allowed to survive.

For objective 2b (above), the brain is removed and incubated in normal saline (with antibiotics) at 37°C. Serial determinations of wet weight and volume are made during the postmortem incubation. In some animals, cerebral blood flow, A-V O_2 and glucose differences and impedence measurements are performed. Brains of survivors are to be examined neuropathologically.

Major Findings: Postmortem brain swelling: preliminary findings indicate significantly smaller water uptake and volume gain of incubated brains in animals which were profoundly hypotensive in the immediate premortem period as compared to normotensive controls. Brains are being examined for differences in postmortem histological changes depending on the presence or absence of premortem hypotension.

Other studies on the metabolic and neuropathological effects of hypotension on the brain are in the formative stages.

Significance: An easily producible and reliable model of the clinical shock syndrome and, hence, cerebral ischemia has been developed without using potent vasomotor drugs or complex neurosurgical procedures. This will be used to study systematically the various physiological, pathological, and biochemical changes associated with shock and cerebral ischemia.

The finding of a significant decrease in the postmortem water uptake and swelling of brains in animals who were hypotensive (and acidotic immediately premortem as compared to normotensive controls) may indicate that rather acute failure of capillary circulation and subsequent disruption of energy-requiring active transport processes cause membrane permeability changes and escape of anaerobic acid metabolites from the brain premortem, with less dysosomosis therefore occurring after death. Whether this process leads to a relative postmortem cellular preservation in profoundly hypotensive animals will be seen after the neuropathology on these brains is complete.

Proposed Course of Project: The hypotensive model will continue to be employed to determine the brain's tolerance to hypotension, the first changes responsible for irreversible damage and the sequence of pathological and possibly biochemical changes produced by cerebral ischemia.

Publications: None.

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Stroke
Intramural Research

Ernst Freese, Chief

Although cellular control mechanisms have now been studied for many years, it is still not known how they enable the cell, at a certain stage of development, to differentiate into a specialized cell type which has lost the capacity to grow. It is also not known how certain differentiated cells can later resume growth when they are brought into a suitable environment. The laboratory investigates control mechanisms involved in differentiation in two biological systems in which large numbers of cells are available for biochemical investigations: the process of sporulation and germination in bacilli and the development of sea urchin eggs after fertilization. In both of these systems the factors controlling ribosomal and messenger RNA synthesis are being isolated at different stages of development, and their effects on RNA polymerase and the synthesis of specific types of RNA are examined. Biochemical mutants have been used to analyze why differentiation is suppressed during cell growth and which biochemical reactions are needed for differentiation. It has been found that at the beginning of development drastic changes occur in the metabolism producing ATP and other metabolites and in the development of bacterial viruses. The commitment of cells to differentiate has been correlated with changes in the transport mechanisms through the cell membrane. Since both the energy source and the mechanism of transport are not known for most substrates, they have been investigated in membrane vesicles for compounds of particular relevance to differentiation. Conditions have been investigated under which the differentiation of developmental mutants can be restored.

1. Control of RNA Synthesis

The rate of RNA synthesis depends on the growth medium and the physiological state of the growing cell. If bacteria are transferred from a rich to a poor medium, a condition very similar to that leading to differentiation, the rate of overall RNA synthesis decreases, owing to a preferential inhibition of ribosomal RNA synthesis. During these conditions a guanosine-3'-di-5'-diphosphate (ppGpp) increases in quantity and decreases again when the cells are transferred to a rich medium. There is actually an inverse correlation between the concentration of ppGpp and the growth rate, implying a physiological function of the compound. Levorphanol, a morphine analog known to inhibit RNA synthesis in pro- and eukaryotes, also causes the accumulation of ppGpp. Ribosomal RNA synthesis, which is stimulated in vitro by a psi factor, if preferentially inhibited by ppGpp. This compound partially inhibits also other enzymes involved in nucleic acid synthesis (IMP dehydrogenase and transport of nucleosides or bases through the cell membrane), indicating that it is mainly responsible for governing the rate of RNA synthesis during growth and differentiation. The attempt to identify the biochemical reaction by which ppGpp is formed has so far not been successful, because the intact cell membrane was required for its synthesis. RNA synthesis seems to be similarly controlled during the embryonic development of sea urchins: at first, ribosomal RNA synthesis is severely curtailed, whereas messenger and nuclear RNA synthesis are active; rapid ribosomal RNA synthesis suddenly commences only during the late gastrula stage. An investigation of the factors controlling the synthesis of specific RNA's is now under way.

2. Use of Biochemical Mutants to Locate the Suppressor of Differentiation on the Biochemical Map

As long as cells grow they do not undergo their differentiation to highly specialized cells, i.e. the process of differentiation requiring the synthesis of specific enzymes is suppressed. For the differentiation process of sporulation this suppression is maintained as long as glucose or other rapidly metabolizable carbon sources are present. In order to locate the suppressor compound(s), mutants have been isolated which cut the Embden-Meyerhof path into two, thereby separating the whole metabolic chart into two disconnected parts. Addition of any carbon source to the upper part suppresses sporulation, whereas addition of any carbon source to the lower part does not suppress sporulation but causes eventual lysis of the cells, apparently due to the lack of glucosamine-6-phosphate which is needed for cell wall synthesis. This result shows that the suppressor of sporulation is made in the upper part of the metabolic chart. By the use of other mutants certain components of this part have been isolated, such as the glycerol pathway or the reactions of glucose-6-phosphate. These mutants are now being investigated to determine which compound suppresses sporulation.

3. Changes in Viral Development during Differentiation

Whereas bacteriophages SP8 or SP01 multiply in exponentially growing cells, producing about 200 new phages after the cells have lysed, this is not so during the developmental period. When bacteria are infected toward the end of exponential growth or later, most cells still lyse but no new phages appear, indicating a drastic change in the cellular metabolism. The lysis depends on continued protein synthesis, since it is blocked by chloramphenicol, whereas it does not depend on RNA synthesis, because it cannot be prevented by actinomycin or rifamycin. This sensitivity to phages can be used as a simple means to distinguish between growing and differentiating cells. The few surviving cells that sporulate incorporate virus DNA in their spores.

4. Mechanism of Membrane Transport and its Changes during the Commitment of Cells to Differentiate

When bacilli have started their differentiation process they become resistant to glucose suppression. At some later time, addition of amino acids also can no longer prevent the continuation of development. Upon dilution in fresh medium, complete spores are made which then can germinate and grow out into new cells. The first commitment of sporulation with respect to glucose has been correlated with the inability of the cells to transport glucose and the inability to induce the PEP-transferase activity that is required for sugar transport. The reason for this lack of inducibility is under investigation. To understand the effect of amino acids, their transport mechanism, which was not known for bacilli, has been studied in membrane vesicles. Amino acids are actively transported into such vesicles in the presence of L-lactate or NADH. Since a strong NADH oxidase uses up the available oxygen in a few seconds, new oxygen had to be provided rapidly. Succinate was effective only when electrons could be coupled from the active succinic dehydrogenase to a later part in the electron transport chain. Inhibitor studies showed that the energy for the amino acid transport is produced in the cytochrome-dependent electron transport chain, but it does not require ATP.

In previous experiments a sporulation mutant had been analyzed which could recover the ability to sporulate in the presence of added acetate. It has now been found that acetate inhibits growth and induces the formation of a membrane protein. The acetate inhibition can be counteracted by glucose, glycerol, oxaloacetate, etc., but not by malate. The new membrane protein is not formed in a succinate dehydrogenase-deficient mutant. In addition, acetate inhibits amino acid transport. These results indicate that acetate may affect either substrate or electron transport mechanisms. The biochemical entities affected as well as the biochemical block of the developmental mutant are under investigation, because they provide an explanation as to how a developmental mutant can be symptomatically cured.

5. The Use of Developmental Mutants for the Identification of Biochemical Reactions Needed for Differentiation

Many developmental mutants have been isolated which cannot sporulate normally. For some of them the biochemical alteration has been identified, such as a number of mutants blocked in the citric acid cycle and others unable to produce cytochrome a or c. These mutants grow normally during the exponential period, but they are unable to sporulate after growth has stopped. This shows that ATP can be synthesized during the exponential growth period independently of the citric acid cycle and only partially dependently on the electron transport chain; these reactions become very important during the differentiation period. Another mutant is blocked in one of the two aspartokinases (lysine insensitive) which have been identified and separated by Sephadex G200 chromatography. Whereas the loss of this enzyme does not prevent growth in a minimal medium (except when lysine inhibits the formation of the other enzyme), it does affect sporulation.

Many mutants having little or no effect on vegetative growth prevent the final burst of spores normally observed after growth has stopped. This shows that differentiation requires a much more delicate control, of counteracting or coupled metabolic reactions, than is necessary for vegetative growth. Many of the mutants are oligosporogenic, i.e. they still produce some spores or give rise to abnormally shaped spores. Such mutants can be compared to human hereditary diseases which also affect an organism only to a sufficiently minor degree that a child is not aborted but is born with a congenital or metabolic disease. Attempts are under way to cure such developmental mutants either by the addition of appropriate compounds or by the supply of specific DNA. Similar experiments are planned in which symptomatic cures of developmental diseases will be attempted in mutant strains of inbred mice.

Serial No. NDS(I)-65 LMB/RN 1208
1. Lab. of Molecular Biology
2. Section on Regulation of Nucleic Acid Synthesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ribonucleic Acid and the Regulation of Cellular Metabolism

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Leonard E. Maroun, Ph.D. and Yoshikatsu Murooka, Ph.D. (Visiting Fellow)

Cooperating Units: Section on Regulatory Cell Physiology, LMB
Department of Genetics, University of Washington, Seattle

Man Years

Total:	3.2
Professional:	2.7
Other:	0.5

Project Description:

Objectives: The nucleic acid content of cells is subject to control by several regulatory mechanisms. The synthesis of certain species of messenger RNA is elicited by the presence or absence of specific metabolites in the cell milieu. The accumulation of ribosomal RNA, however, does not respond to the availability of specific nutrients but responds, rather, to the fitness of the medium for growth and the growth rate of the cell. Transfer RNA, on the other hand, responds neither to specific nutrients nor to the growth rate but is constant under most conditions of growth. The details of the mechanisms underlying these observations are at present obscure and are the subject of this project. We have concentrated on three aspects of these control mechanisms: 1) Control of ribosomal RNA synthesis in growing and nongrowing microbial cells. 2) The cell-free transcription of ribosomal RNA. 3) Control of ribosomal RNA during early embryogenesis.

Methods Employed: Standard biochemical techniques are employed for the purification of RNA, DNA and enzymes. Analysis of complex mixtures of RNA are performed by chromatography on Sephadex G-100 or methylated albumin or polyacrylamide gel electrophoresis. Messenger RNA is estimated by molecular hybridization with homologous DNA. The rates of synthesis of individual RNA species are determined from the kinetics of incorporation of radioactive precursors into the species.

Major Findings: Last year's Project Report summarized our results on the types and amounts of RNA synthesized in microbial cells during normal growth and under two nongrowth conditions: amino acid deprivation and step-down transition from rich to poor media. These results indicated that microorganisms regulate the synthesis of ribosomal RNA and messenger RNA independently. Under the two nongrowth conditions examined, ribosomal RNA synthesis was severely curtailed, while messenger RNA synthesis was only slightly abated. Furthermore, our results indicated that cells adjust and maintain their RNA content during normal growth by the same mechanisms that effect a virtual complete cessation of ribosomal RNA synthesis during the nongrowth culture conditions. Our working hypothesis was that this regulation of the rate of ribosomal RNA synthesis was mediated by the guanosine tetraphosphate, ppGpp.

1. Regulation of ppGpp. The hypothesis that the regulation of ribosomal RNA synthesis is mediated by guanosine tetraphosphate suggests that the intracellular concentration of the nucleotide should bear some constant relationship to the rate of rRNA synthesis. We have measured the intracellular levels of ppGpp and rRNA in cells cultured at different growth rates. The results of these studies show that there exists an inverse linear relationship between the RNA content and ppGpp levels in growing cells. This kind of relationship would be expected if ppGpp acted as an inhibitor of rRNA synthesis. Furthermore, it was found that under several nongrowth conditions (amino acid and carbon deprivation and stepdown transitions from rich to poor media) the ppGpp level was elevated and rRNA synthesis was curtailed, while under other nongrowth conditions (phosphate, uracil, and guanosine deprivation) the ppGpp concentration remained low and rRNA synthesis continued. The conclusion drawn from these studies is that under all of the conditions examined, the behavior of the intracellular ppGpp and the rate of rRNA synthesis were consistent with the idea that the nucleotide inhibits the synthesis of rRNA.

2. Cell-free studies of rRNA synthesis. In order to further pursue the regulation of rRNA, we undertook a study of the *in vitro* synthesis of rRNA. Ribosomal RNA genes constitute about 0.3% of the total genetic material of *Escherichia coli*. Yet in the growing cell about 40% of all RNA synthesized is ribosomal RNA. To a first approximation, then, rRNA genes are transcribed by the RNA polymerase about 150 times more frequently than the average gene. However, we, as well as others, have observed that when *E. coli* DNA is used as a template in the usual RNA polymerization reaction with the RNA polymerase of *E. coli*, less than 0.3% of the RNA synthesized is rRNA. Recently Dr. A. Travers described an activity in crude extracts of *E. coli* that is required for the transcription of rRNA. We have found thatthis activity can be resolved into at least two protein factors, both of which stimulate rRNA transcription. The evidence thus far gathered by this laboratory indicates that both protein factors have a molecular weight of approximately 100,000 daltons and activate RNA synthesis by binding to specific sites on the DNA rather than affecting the RNA polymerase directly.

The binding to DNA is not inhibited by the presence of ppGpp, although the presence of this nucleotide specifically inhibits the transcription of rRNA cistrons. Our working hypothesis is that the nucleotide ppGpp specifically interacts with the RNA polymerase and subtly alters the configuration of the protein so that its activity can no longer be stimulated by the two protein factors we have described.

3. Regulation of RNA synthesis in higher organisms. With a detailed description of the regulation of ribosomal RNA in microorganisms imminent, we have directed some of our attention to the regulation of RNA synthesis in higher organisms. For this study we have chosen the sea urchin, because the early embryonic development from the fertilized egg shows many evidences of regulation of rRNA synthesis. In particular, we are studying a) the mechanism by which rRNA synthesis is suppressed in the early development of the sea urchin embryo while messenger RNA and DNA-like RNA synthesis continues, b) the events leading to the commencement of rapid rRNA synthesis at the gastula stage, and c) the regulation of rRNA in the mitochondria. These studies have just been initiated and results are not yet forthcoming.

Significance to Bio-medical Research and the Program of the Institute: Controls of nucleic acid synthesis in bacterial systems represent examples of regulatory mechanisms which are more amenable to experimental investigation than those of the more slowly-growing mammalian systems. It is anticipated that information obtained from these simpler systems will help form a basis upon which an investigation of the more complex regulatory mechanisms of higher organisms can be built and eventually an understanding and control of disease in these systems can be obtained.

Proposed Course of Project: 1. To investigate further the cell-free synthesis of ribosomal RNA, including the role of the two protein factors and the inhibition of rRNA synthesis by ppGpp. 2. To investigate the nature of the three separable RNA polymerases found in sea urchin eggs and embryos and the cytological localization of these enzymes. 3. To investigate in whole embryos the synthesis of rRNA during the course of development.

Honors and Awards: None

Publications:

Lazzarini, R. A. and Winslow, R. M.: The regulation of RNA synthesis during growth rate transitions and amino acid deprivation in *E. coli*. *Cold Spring Harbor Sympos. Quant. Biol.* 35: 383-390, 1970.

Lazzarini, R. A. and Dahlberg, A.: The control of RNA synthesis during amino acid deprivation in *E. coli*. *J. Biol. Chem.* 246: 420-429, 1971.

Lazzarini, R. A., Cashel, M., and Gallant, J.: On the regulation of ppGpp levels in stringent and relaxed strains of *E. coli*. *J. Biol. Chem.*, in press.

Winslow, R. M.: The consequence of the *rel* gene during a glucose to lactate downshift in *Escherichia coli*: The rates of RNA synthesis. *J. Biol. Chem.*, in press.

Serial No. NDS(I)-65 LMB/DB 1244
1. Lab. of Molecular Biology
2. Section on Developmental
Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Control Mechanisms and Differentiation

Previous serial number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Martin D. Diesterhaft, Ph.D., Michael L. Hampton, Ph.D., Wilhelmus N. Konings, Ph.D. (Visiting Fellow), Chandan Prasad, Ph.D. (Visiting Fellow), Norman L. Richards, Ph.D., Ching-ju W. Sheu, Ph.D. (Visiting Fellow), Eugene L. Speck, M.D., Ph.D.

Cooperating Units: None

Man Years

Total:	8.6
Professional:	7.1
Other:	1.5

Project Description

Objectives: The transition from growing into differentiating cells is similar in micro- and macroorganisms. In both cases the biochemical reactions required for this transition and for the commitment of cells to continue differentiation are not known. Our project has been concerned with these biochemical reactions in Bacillus subtilis, because this microorganism can be rapidly grown, biochemical and developmental mutants can be easily isolated, and a genetic analysis is feasible by transformation or transduction. In the course of these studies it became clear that some physiological phenomena, such as the transport of amino acids into cells, are not even understood in normal growing cells.

Methods Employed: Mutants deficient in development or in specific enzymes were isolated by mutagenizing cells with ethylmethanesulfonate, selecting mutants with staphcillin, and identifying them by replica plating. The production of spores was measured by the formation of heat-resistant particles. Membrane protein patterns were analyzed on acrylamide gels and radioactive patterns were determined by cutting the gels with a specially designed gel cutter. Bacterial membranes were obtained after protoplasting of cells in sucrose and lysing them by rapid dilution. Maximal incorporation of amino acids into membrane vesicles was found under conditions under which the reaction tubes containing 5 mm long stirring bars were placed on a magnetic stirrer and oxygen was blown into the tubes throughout the reaction time.

Major Findings: 1. Biochemical location of the suppressor of sporulation. When bacilli are grown in a nutrient sporulation medium containing excess glucose or other rapidly metabolizable carbon sources, the sporulation process remains permanently suppressed. Since the carbon from glucose can enter all biochemical pathways, it would be difficult to identify the suppressor of differentiation. A mutant lacking phosphoglycerate kinase has therefore been isolated which effectively cuts the metabolic chart into two parts, an upper and a lower subdivision. In this mutant glucose still suppresses sporulation, whereas malate entering the lower metabolic subdivision does not prevent the initiation of sporulation, although it later causes lysis of the cell, presumably due to glucosamine deficiency. The suppressor of sporulation, therefore, seems to be located in the upper metabolic subdivision. To narrow down its location, other mutants, lacking phosphoglucomutase, phosphoglucoisomerase, and glucose-6-phosphate dehydrogenase, have been isolated and double and triple mutants have been obtained. It was surprisingly found that double mutants lacking phosphoglucose isomerase and glucose-6-phosphate dehydrogenase lysed very early after they had accumulated high quantities of glucose-6-phosphate. The mechanism of this phenomenon is of interest, not only for sporulation, which obviously was prevented by the lysis, but also for human glucose-6-phosphate dehydrogenase deficiencies which, in the presence of certain compounds such as primaquin, cause hemolysis.

2. The effect of acetate on growth, sporulation, and synthesis of membrane protein. Whereas most sporulation mutants could not be cured with respect to sporulation by any added compound, one mutant responded to acetate when it was added during the exponential period. Consequently, the effect of acetate on growth and sporulation was examined. 200 mM acetate partially inhibit growth and cause the accumulation of a new protein in the cell membrane fraction. This protein is not made in mutants lacking succinic dehydrogenase, suggesting that it is part of the electron transport chain. However, acetate also inhibits the uptake of radioactive amino acids into cells or membrane vesicles, suggesting the alternate possibility that acetate interferes directly with the carrier protein of amino acid transport. The new membrane protein, formed in the presence of acetate, may therefore be a consequence of derepression with respect to a compound whose uptake or production is inhibited by acetate. The relation of this phenomenon to the curing of sporulation is being investigated.

3. Amino acid transport in membrane vesicles. In order to examine the change of transport properties of bacterial cells during commitment to differentiation, the transport of sugars and amino acids through membranes has been examined. We had previously reported that the transport of sugar by glucose-phosphoenolpyruvate transferase decreased toward the end of growth, with the result that the committed cells were refractory to the entrance of glucose during the developmental process. In order to perform similar studies for amino acid transport, membrane vesicles of exponentially growing cells were isolated and the energy requirement for the transport was first studied. It was found that NADH and NADPH allowed only a limited amount of amino acid uptake, due to the rapid exhaustion of oxygen in even a very small reaction volume. Only if oxygen was supplied continuously and rapidly could a maximum rate of amino acid incorporation be observed. This incorporation was clearly linked to the electron transport chain and studies are now under way to establish at which place of the electron transport chain the energy for transport (conformational change of carrier protein) is produced. An important tool for this study was our discovery that amino acid transport could also be stimulated by ascorbate plus phenazine methosulfate, which enters the electron transport chain at some place below cytochrome b. The relation of these findings to differentiation is indicated by the preliminary observation that amino acid transport decreases drastically toward the end of exponential growth.

4. Analysis of mutants lacking cytochrome a or c. Mutants lacking cytochrome a or c, which had been previously isolated, were unable to sporulate normally. Surprisingly, however, they were able to produce ATP for several hours after growth had stopped. This result showed that neither cytochrome a nor cytochrome c are essential for the production of ATP in the electron transport chain, which must contain a bypath of these two cytochromes. In fact, a cytochrome o has been observed, which may be an alternate terminal cytochrome oxidase. Although ATP can still be maintained, these mutants cannot sporulate, indicating that cytochrome a- or c-deficient mutants are blocked more importantly in some other metabolic reaction such as the uptake of amino acids examined above. This phenomenon, which places a new emphasis on the importance of cytochromes for differentiation, is under further investigation.

5. Developmental significance of aspartokinase. Aspartokinase is the first enzyme in the synthesis of several branched amino acids: methionine, threonine, isoleucine, and lysine. During sporulation this enzyme activity is also necessary for the synthesis of dipicolinic acid, which is incorporated in large quantities into the spore cortex. In B. subtilis we have detected two aspartokinases, one of which is inhibited by lysine and the other stimulated by leucine. The lysine-inhibited enzyme decreases toward the end of growth, so that it would not be capable of producing the required dipicolinic acid during sporulation. The importance of the lysine-insensitive enzyme for differentiation is shown by the fact that mutants lacking this enzyme can still grow vegetatively but are unable to sporulate. There-

fore, the cell seems to maintain two aspartokinases not only because they control the synthesis of different amino acids but also because the need for biochemical control of these enzymes changes during differentiation.

Significance to Bio-medical Research and the Program of the Institute: Some of the developmental mutants analyzed in B. subtilis are blocked in enzymes that are deficient also in certain human hereditary diseases. The difficulty encountered in restoring normal sporulation indicates the enormous problem which a hereditary disease poses for differentiation. In most cases avoidance of the disease by genetic counseling will be preferable to attempts of partially curing it. Nevertheless, the biochemical studies with microorganisms show that differentiation of certain mutants can be restored under certain carefully controlled conditions; hereditary diseases lacking these enzymes may therefore be treatable under similar conditions also in man.

Proposed Course of Project: Further mutants will be isolated in order to determine in more detail the phenomenon of sporulation suppression. These studies will also yield results about the possibility to cure developmental mutants with respect to sporulation and the reason why glucose-6-phosphate dehydrogenase deficiency renders cells sensitive to lysis. Following the characterization of amino acid transport in exponentially growing cells, the change of this transport in sporulation and in cytochrome mutants will be analyzed. Realizing the rare opportunity of curing a sporulation mutant, the phenomenon of acetate on sporulation and on substrate transport will be analyzed in biochemical detail.

Honors and Awards: Invited speaker at symposia:

1. Symposium on Genetics of Industrial Microorganisms, Prague, Czechoslovakia, August 1970, "Early events in sporulating Bacillus subtilis"

2. American Society for Microbiology, May 1971, Symposium on Cellular Differentiation, "Sporulation in Bacilli"

Publications:

Freese, E., Klofat, W., and Galliers, E.: Commitment to sporulation and induction of glucose-phosphoenolpyruvate-transferase. Biochim. Biophys. Acta 222: 265-289, 1970.

Sugae, K. and Freese, E.: Requirement of acetate and glycine (or serine) for sporulation of Bacillus subtilis. J. Bact. 104: 1074-1085, 1970.

Konings, W. N. and Freese, E.: L-Serine transport in membrane vesicles of Bacillus subtilis energized by NADH or reduced phenazine methosulfate. FEBS Letters 14: 65-68, 1971.

Freese, E.: On the genetic effects of Isoniazid and other hydrazides. Environmental Mutagen Society Newsletter 4: 8-9, 1971.

Freese, E.: Molecular mechanisms of mutations. In Hollaender, A. (Ed.): Chemical Mutagens: Principles and Methods for Their Detection. New York, Plenum Press, 1971, pp. 1-56.

Freese, E.: Structure-activity considerations in potential mutagenicity. In Epstein, S. and Lederberg, J. (Eds.): Drugs of Abuse, Genetic and Other Chronic Nonpsychiatric Hazards. Washington, D. C., U. S. Government Printing Office, 1971, in press.

Freese, E., Sugae, K., and Klofat, W.: Early events in sporulating Bacillus subtilis. In Proceedings of Symposium on Genetics of Industrial Microorganisms, Prague, Czechoslovakia, Aug. 24-29, 1970. Prague, Academia, in press.

Serial No. NDS(I)-68 LMB/RC 1552
1. Lab. of Molecular Biology
2. Section on Regulatory Cell Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Integrative Control of Macromolecular Synthesis

Previous Serial Number: SAME

Principal Investigator: Michael Cashel, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Section on Regulation of Nucleic Acid Synthesis, LMB
Department of Genetics, University of Washington, Seattle
Biological Laboratories, Harvard University, Cambridge, Mass. and Laboratory of Molecular Biology, Medical Research Council, Cambridge, England

Man Years

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objectives: The broad objective of the project is to understand the mechanisms by which cells maintain a balanced composition under diverse physiological growth conditions. As described in previous annual reports, experimental attention is focused on the mechanisms by which the accumulation of guanosine-5'-diphosphate-2'(or 3')-diphosphate (ppGpp) mediates restriction of RNA synthesis when protein synthesis is limited for lack of a complete array of aminoacylated tRNA species.

Methods Employed: In addition to those described in earlier reports, the following techniques have been used: protein and nucleotide fluorescence, acrylamide gel electrophoresis of proteins (denatured), as well as in situ assays of RNA polymerase in gels, equilibrium dialysis, and metabolic turnover studies on nucleotidyl phosphates.

Major Findings: 1. The effect of ppGpp on RNA polymerase transcription. The addition of ppGpp to RNA polymerase reaction mixtures under a variety of assay conditions results in a limited inhibition of total transcriptional activity. The inhibition observed occurs with physiological levels of ppGpp relative to GTP concentrations. With native DNA templates, the maximum inhibition is about 30%, whereas inhibition rises to about 50% with denatured DNA. The limited inhibition is seemingly independent of physiological initiations, since similar inhibition is observed with core enzymes lacking sigma factor, and with DNA templates purposefully nicked or broken with DNase I. The kinetics of ppGpp inhibition show a mixed type of competitive inhibition with GTP, an apparent Ki (ppGpp) of 0.05 mM, and no dependence on any other component of the assay mixture. The inhibitor does not lead to the formation of small RNA chains whose elongation is stopped short by incorporation of ppGpp on the growing end of the RNA chain. This result is confirmed with studies showing the lack of incorporation of labeled ppGpp into RNA, even when DNA-polymerase-RNA complexes are recovered and assayed. The inhibition by ppGpp also does not appear to be mediated by RNA chain termination: the compound is active in the presence of rho factor as well as when chain termination occurs nonphysiologically as in the presence of high osmotic strength. The evidence does implicate a selective effect of ppGpp on the initiation of RNA transcription. Studies with purine 5'-triphosphates labeled in the gamma position show that ppGpp mediates the abolition of about half of the RNA chains initiated with GTP while not affecting RNA chains initiated with ATP. The mechanism by which half of the GTP starts are inhibited is obscure, but might involve a chemical specificity for the penultimate nucleotide in the start sequence. Pyrophosphate exchange studies bearing on this possibility are in progress.

2. Effect of ppGpp on RNA polymerase transcription of ribosomal RNA. Recently Travers, Kamen and Schlief have demonstrated that a protein (psi greatly stimulates the ability of RNA polymerase to transcribe RNA whose hybridization to DNA is competed by ribosomal RNA. It is supposed that psi mediates ribosomal RNA transcription in vivo, since the frequency of ribosomal genes in DNA is about one hundred times less frequent than the rate of cellular transcription of rRNA. The addition of ppGpp to transcription assays indicates that a marked, preferential inhibition of rRNA-like RNA transcription occurs. This result is taken as a preliminary indication that ppGpp can function as a mode of restricting ribosomal RNA synthesis. In addition, the activity of RNA viral replicase (QB) is thought to also be subject to regulation by amino acid availability. Travers, Kamen and Schlief have shown that two of the host-derived subunits of QB replicase have psi activity. Again in collaboration, measurements of ppGpp inhibition indicate that the compound inhibits stimulation of rRNA-like RNA transcription by these subunits as well as the transcription of poly C templates by the intact viral enzyme. Mechanism studies again implicate interference with GTP initiation processes.

3. The interaction of ppGpp with RNA polymerase protein. RNA polymerase both containing or lacking sigma factor has been purified to homogeneity as judged by gel electrophoresis. Studies of binding of ppGpp to the enzyme by column or membrane equilibrium dialysis techniques show that ppGpp binds to core enzyme. The binding constant is the same order of magnitude as GTP and is unaffected by binding of template to the enzyme. DNA itself does not bind ppGpp. RNA polymerase binds one molar equivalent of ppGpp at saturating ppGpp

concentrations. Fluorescent studies by Wu and Goldthwaite have indicated that RNA polymerase possesses two classes of nucleotide binding sites. Analogous studies with ppGpp apparently localize its site of binding as identical with the initiation site of the enzyme. ppGpp binding occurs in the absence of magnesium and quenches the fluorescence of a tryptophan residue, as does initiating purine nucleotides and rifampicin. Pyrimidine nucleotides do not bind to this site.

4. The production of ppGpp in relaxed mutants. In collaboration with Drs. Lazzarini and Gallant, measurements of ppGpp accumulation under a variety of growth conditions were made with normal cells and with mutants whose RNA accumulation is independent of amino acid availability, so-called relaxed mutants. Such mutants can regulate their rates of rRNA synthesis during carbon source deprivation. These studies confirm earlier measurements of the ability of relaxed mutants to accumulate ppGpp when the cells do restrict rRNA synthesis, but not during amino acid deprivation. Furthermore, the basal levels of ppGpp in normal and mutant strains are inversely correlated with growth rates and rRNA accumulation, suggesting that the compound functions even at relatively low intracellular concentrations similarly in both normal and mutant cells.

5. Biological synthesis of ppGpp. Studies in progress indicate that ppGpp labeling with ^{32}P orthophosphate does not occur in either crude extracts or in S30 preparations capable of poly U translation and inhibition by fusidic acid. Cells made permeable to nucleotides by plasmolysis, synthesize large amounts of ppGpp, but this capacity is abolished by the addition of membrane-dissolving compounds (toluene or Triton). Selective degradation of the pulse-labeled phosphates of ppGpp reveal that the 2' or 3' beta phosphate is metabolically labile, while the alpha phosphate is stable. This observation eliminates the possibility of biological synthesis by successive phosphorylations with gamma nucleotidyl phosphates. This is confirmed with plasmolysed cell studies; also in such systems alpha-labeled UTP, CTP, GTP, and ATP do not label ppGpp. The biological route of ppGpp synthesis remains obscure.

Significance to Bio-medical Research and the Program of the Institute: In general, it is often cited that an understanding of the means by which normal cells regulate their components is essential for a rational approach to abnormal cell physiology. In particular, recent descriptions of mammalian muscle cell physiology during hypertrophy (achieved by removal of a synergistic muscle) and atrophy (denervation) closely parallel the alterations observed in E. coli during amino acid supplementation and starvation, respectively. Under both conditions, integrative control of RNA accumulation and protein accumulation is displayed, as well as alterations in nucleoside transport, certain aspects of nucleotide metabolism, and protein breakdown.

Proposed Course of Project: This project will be continued in NICHD, transfer effective May 2, 1971. To speed knowledge of the biological effects of ppGpp, the compound has been shipped to or given to a number of investigators requesting samples: Dr. J. T. Bonner (Princeton University), Dr. A. Goldberg (Harvard Medical School), Dr. J. Preiss (University of California, Davis), Dr. G. Zubay (Columbia University), Dr. G. Edlin (University of California, Davis), Dr. W. Rutter (University of California, San Francisco), Dr. D. Chambers (Columbia University), Dr. I. Pastan (NCI), Dr. P. Fromageot (CNRS, Gif-sur-Yvette, France), Dr. A. Travers (Medical Research Council, Cambridge, England), Dr. J. Irr (Marquette University), Dr. G. Tomkins (University of California, San Francisco), Dr. M. Singer (NIAMD), Dr. O. Miller (Oak Ridge National Laboratory), Dr. D. Schlessinger (St. Louis University), Dr. J. Hurwitz (Albert Einstein College of Medicine), Dr. R. Guesteland (Cold Spring Harbor Laboratory), Dr. J. Ozer (NHLI), Dr. R. Lazzarini (NINDS). The areas of inquiry involve effects on DNA-dependent protein synthesis, RNA control, nucleoside transport, and occurence in mammalian cells.

Honors and Awards: None

Publications:

Cashel, M.: Inhibition of RNA polymerase by ppGpp, a nucleotide accumulated during the stringent response to amino acid starvation in *E. coli*. *Cold Spring Harbor Sympos. Quant. Biol*. 35: 407-413, 1970.

Travers, A., Kamen, R., and Cashel, M.: The *in vitro* synthesis of ribosomal RNA. *Cold Spring Harbor Sympos. Quant. Biol*. 35: 415-418, 1970.

Gallant, J., Irr, J., and Cashel, M.: The mechanism of amino acid control of guanylate and adenylate biosynthesis. *J. Biol. Chem.*, in press.

Serial No. NDS(I)-71 LMB/DB 1886
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Developmental Cytology

Previous serial number: None

Principal Investigator: Elisabeth B. Freese, Ph.D.

Other Investigators: Yong K. Oh, Ph.D. (Visiting Fellow)

Cooperating Units: None

Man Years

Total:	2.4
Professional:	1.7
Other:	0.7

Project Description

Objectives: Differentiation entails a sequential development from vegetatively growing cells into highly differentiated cells. In most laboratories differentiation is studied either at the biochemical or at the cytological level, making it difficult to decide which biochemical reaction corresponds to which cytological change of development. It is our aim to obtain a close correlation between the two types of investigations and thus to unravel which biochemical reactions are needed for each developmental step. Such an analysis can be performed in depth in a very simple organism, such as the sporulation of Bacillus subtilis, whose cells grow rapidly and can be genetically transformed, and for which mutants can be easily isolated. A correlation between cytological and biochemical events is particularly feasible in developmental mutants that are blocked, or can be artificially arrested, at certain stages of development.

Methods Employed: For electron microscopy the standard strain and developmental mutants were grown to different stages of development, samples were centrifuged, fixed by glutaraldehyde, stained with osmium tetroxide and uranyl acetate, embedded in Epon, and after sectioning, stained by lead citrate. The sections were investigated in a Zeiss EM 9-52 electron microscope. Developmental mutants were induced by ethylmethanesulfonate and both purified and converted to double mutants by DNA transformation.

Major Findings: 1. Developmental block of cytochrome-deficient mutants. Mutants lacking cytochrome a or both cytochromes a and c were able to grow exponentially in nutrient sporulation medium and maintained ATP for several hours after growth had stopped. However, all of these mutants could not sporulate normally. If the decline of the ATP concentration were the major cause for the lack of sporulation, the different mutants should be arrested at different stages of development. Electron microscope investigation showed, however, that all mutants were unable to form a prespore septum, which is the first cytologically recognizable development leading to the formation of spores. Since this septum is normally made one to two hours after growth has stopped, the lack of sporulation in these mutants results from some other deficiency rather than the lack of ATP. For example, the transport of amino acids, which depends on an intact cytochrome system, may be defective in these mutants.

Instead of forming a normal septum, the cytochrome mutants display an exuberant growth of membrane vesicles, which indicates that the control of membrane synthesis has gone wrong. The reasons for this abnormality are now under investigation.

2. Investigation of the suppression of sporulation in developmental mutants. A mutant lacking phosphoglycerate kinase could still sporulate almost normally in nutrient sporulation medium, but its sporulation was suppressed by both glucose and malate. Electron microscope investigations revealed that glucose suppressed the start of sporulation, whereas in malate the first developmental processes apparently could proceed but then lysis of the sporulating mother cell ensued. These findings indicate that the suppressor of sporulation is produced in the upper metabolic subdivision into which glucose, fructose, and other carbon sources can enter. In order to locate this suppressor more precisely, double mutants are now being isolated lacking both phosphoglycerate kinase and either the enzyme for glucosamine synthesis or that for glycerol synthesis. Whereas glucosamine had been shown to be necessary for the synthesis of spore cortex synthesis in glucosamine-requiring mutants, the importance of this compound earlier in sporulation can only be assessed in such double mutants.

Significance to Bio-medical Research and the Program of the Institute: The mutants investigated here show that certain biochemical reactions are much more important for differentiation than for vegetative growth. Many hereditary human developmental abnormalities are also caused by the lack of enzymes which apparently are more important for differentiation. However, if one wants to effect a cure of such a disease, it is not sufficient to know which enzyme is altered, but one must find out why this alteration produces an abnormal development. Our studies attempt to unravel this cause for microorganisms in the expectation that essentially the same explanation will hold for human mutants.

Proposed Course of Project: The involvement of cytochromes in the control of membrane synthesis, and the relationship to cell division will be analyzed by a functional correlation between biochemical and electron microscopic studies in developmental mutants.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Associate Director, Collaborative and Field Research
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Through October 31, 1970, leadership of this program area was provided on an acting basis by Dr. Eldon L. Eagles, Deputy Director, NINDS. On November 1, Dr. Warren V. Huber was appointed Associate Director. Dr. Huber was formerly Project Director of the American Neurological Association's Joint Committee for Stroke Facilities, and had served as Chief of Neurology in the Veteran's Administration's Department of Medicine and Surgery. Dr. Huber's appointment marked a re-emphasis of the role of collaborative, directed, and field-type research in the overall effort to accomplish the scientific objectives of this Institute. The intent of this report is not to encapsulate items of information found in the separate branch summaries but to provide an overview of the significant accomplishments and problems of the Institute's Collaborative and Field Research programs during Fiscal Year 1971, and to look briefly, though broadly, toward the future of these programs.

PROGRESS AND ACCOMPLISHMENTS, FY 1971

Increased funds provided by the President's budget and congressional supplements permitted orderly program expansion in epilepsy, funding for a pilot study of cerebral death, and sufficient support to prevent dislocation in other programs. Without these increases significant program opportunities would have been lost.

The Special Projects Branch continued to pursue studies of neurologic disorders, concentrating on controlled clinical studies for the evaluation of two experimental anticonvulsants and one marketed agent. These efforts have demonstrated the value of blood level determinations as an adjunct to anticonvulsant therapy and established methodologies for further anticonvulsant studies. The successful transition of Epilepsy Abstracts from a fully supported Institute publication to a partially subsidized periodical available by subscription has demonstrated that periodical's scientific merit and value. The completion of the evaluation of the 15-year field follow-up of head injured veterans of the Korean campaign, and the evaluation of data from Phase I of the Head Injury Model Construction Program, mark the conclusion of two important aspects of the Institute's directed Head Injury Program. Funds must now be obtained and leadership recruited for continued efforts in this important program area.

The process of planning and developing a collaborative study of the criteria of cerebral death was accomplished by staff of the Office of the Associate Director and the Head Injury Section of the Special Projects Branch. With expert guidance from an ad hoc advisory committee, an acceptable protocol was developed and a coordinator and collaborators were selected. Contracts for the study will be awarded before July 1, 1971.

Results of controlled and limited studies using the assembled data base have continued to come in to the Perinatal Research Branch. Subject area task forces, in collaboration with the Epidemiological and Statistical Advisory Committee, are redirecting staff effort and attention to larger, more significant statistical analyses to identify major trends, developmental patterns, and risk factors. Interesting and significant studies of the influence of infectious diseases in pregnancy and childhood upon the development of the child's central nervous system (CNS) continue.

A multi-institutional study to determine the feasibility of developing an effective sensory prosthesis for the blind has been launched. Currently, the study is focused on the problems of direct stimulation of visual centers of the brain, including the safety thereof, and the reproducibility of stimulation results. The future success of this project is enchanced in large part by the careful, expert, and conscientious project direction being provided by the Chief, Laboratory of Neural Control, Intramural Research, and his assistant.

Epidemiologic studies of a selected group of acute and chronic nervous system disorders continue. Short term studies, particularly of multiple sclerosis, sub-acute sclerosing panencephalitis, and CNS tumors have pointed the way toward a better understanding of the etiology of these diseases.

Collaboration with scientists working in a broad range of the basic biological sciences and in clinical medicine has improved considerably our understanding of the mechanisms and etiology of slow, latent and temperate virus infections. Successful transmission of two sub-acute and chronic degenerative diseases of the nervous system from man to primates has led to the implication of suspect viral agents--a significant step in the march toward understanding the etiology and thereby, hopefully, being able to successfully treat or prevent such types of central nervous system disease.

PROBLEMS - FY 1971

Despite the successes and progress of the past year there are problems affecting program development which remain to be solved.

Tight controls on full-time and permanent employment restricts the recruitment of staff needed to implement and develop important new programs. This has necessitated the most careful allocation of positions as vacancies occur. Only through this mechanism can needed and important shifts in priority be implemented.

Head and spinal cord injury has been recognized as a serious problem for several years. Despite generous Extramural support and a vigorous Intramural program, important scientific questions remain unanswered; these must be answered if the goal of improved treatment, coupled with improved prevention, is to be attained. Directed collaborative studies aimed at elucidating the pathophysiologic responses of brain and spinal cord tissues to trauma are urgently needed. Until additional funds are available and adequate leadership can be found--and the two needs are interrelated--studies in this important problem area cannot be developed.

The significant FY 1972 budget decrease that has been imposed on the Perinatal Research Branch (PRB) makes it exceedingly difficult to accomplish two important tasks. The first is an orderly completion of the Collaborative Perinatal Project. As a result of the cut, important activities in follow-up, sample maintenance, and quality control at the collaborating institutions and at the coordinating center in the PRB will be curtailed. The second task which must be foregone is the planning for research projects on neurological diseases of childhood; preliminary results of the Collaborative Perinatal Project indicate these would be productive. Although an orderly decrease in budget support and effort by the PRB was planned as the study phased out, the immediate and drastic decrease at this time is quite damaging.

The Collaborative and Field Research Programs of NINDS are, by their nature, heavily dependent upon continuing and active biostatistical participation. The need for biostatisticians in these varied programs goes deeper than simple part-time consultation. To be effective, the statistical staff must be part of the research teams and relate closely to them. Scientific program leaders, charged with the responsibility of promptly producing evidence of research results to fully collaborate with scientists outside of NINDS, must have the explicit and constant control of the processing and analysis of the data upon which they rely. Without such control they have experienced an inability to discharge these responsibilities promptly and effectively. Therefore, it is imperative that such a realistic relationship with the Office of Biometry, NINDS, be established.

LOOKING AHEAD

Fiscal Year 1972 promises to be a difficult year in terms of availability of resources to move forward with existing and planned programs. Budget cuts will effect the Epilepsy, Head Injury, and Cerebral Death Programs as well as the Collaborative Perinatal Project. Considerable resourcefulness will be required to maintain programs which have been so laboriously built and organized. In no way will it be possible to mount new programs in stroke research, in disorders of communication, or to pursue significant head and spinal cord injury research.

As data collection for the Collaborative Perinatal Project moves toward completion, and is supplanted by less expensive, but promising, investigations of neurologic disorders of childhood, funding required by the Perinatal Research Branch will diminish. It is anticipated that this will permit the resumption of active, directed, collaborative research in the field of head and spinal cord injury, and the commencement of similar efforts in the fields of communicative disorders and stroke, with only relatively minor increases in the overall funding level over the next five years.

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